UNIVERSITY OF ALBERTA

THE EFFECT OF A SHORT-TERM, FAT-REDUCED DIET ON SERUM LUTEINIZING HORMONE AND CORTISOL LEVELS AND SALIVARY PROGESTERONE LEVELS IN YOUNG WOMEN

BY

ALEXANDRA C. SANDERMAN ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science.**

FACULTY OF **PHYSICAL EDUCATION AND RECREATION**

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ABSTRACT. The purpose of the present study was to determine if a 5-day, fat-reduced diet affects mid-follicular phase serum LH pulsatility, serum cortisol concentrations and salivary luteal phase progesterone profiles in young eumenorrheic women. Ten (10) non-athletic women between the ages of 20 -23 were subjected to a 5-day,10% fat diet. Hormonal indices were compared to those of the previous menstrual cycle during which participants consumed their regular diet.

No significant differences were noted in mid-follicular phase LH pulse frequency (5.6 ± 0.2 pulses/8 hrs *vs* 5.6 ± 0.3 pulses/8 hrs), pulse amplitude 1.0 ± 0.2 mIU/ml *vs* 1.1 ± 0.2 mIU/ml), mean LH concentrations (2.1 ± 0.4 mIU/ml *vs* 1.9 ± 0.4 mIU/ml) and LH area-under-the-curve (55.8 ± 10.7 mIU/ml•min *vs* 55.5 ± 12.7 mIU/ml•min. Mean cortisol concentrations did not differ significantly (250 ± 27.9 nmol/L *vs* 240 ± 16.1 nmol/L). Cortisol area-under-the-curve also did not differ (2030 ± 8.2 nmol/L•hr *vs* 1910 ± 126.9 nmol/L•hr). No significant differences were noted between mean luteal phase progesterone concentrations (711 ± 49.3 pmol/L *vs* 599 ± 79.2 pmol/L) or luteal phase progesterone area-under-the-curve (11,448 ± 916.5 pmol/L•day *vs* 9502 ± 980.7 pmol/L•day).

Short-term reductions in dietary fat did not significantly change the hormonal mileau of young eumenorrheic women.

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LIST OF ABBREVIATIONS

ACTH - Adrenalcorticotropin Hormone
AM - Amenorrheic
BMI - Body Mass Index
CHO - Carbohydrate
CRH - Corticotropin Releasing Hormone
EFP - Early Follicular Phase
EU - Eumenorrheic
FSH - Follicle Stimulating Hormone
GnRH - Gonadotropin Releasing Hormone
HDL - High Density Lipoprotein
HPA - Hypothalamic Pituitary Adrenal
HPO - Hypothalamic Pituitary Ovarian
LDL - Low Density Lipoprotein
LFP - Late Follicular Phase
LH - Luteinizing Hormone
MFP - Mid Follicular Phase
Oligo - Oligomenorrhea

CHAPTER ONE

INTRODUCTION

Overview

Sociocultural factors which portray leanness as the health and beauty ideal have pressured the 90's woman in the attainment of unrealistic weight goals (Rodin & Larsen 1992). The 1990 Health Promotion Survey reported that 67% of Canadian women desired weight loss to some extent while 39% were, at that time, undergoing a weight reduction program (Health & Welfare Canada 1993). Based on Body Mass Index (BMI) standards, 37% of these dieting women were considered within acceptable weight range while 8% were rated as underweight. These results emphasize the increased dissatisfaction with body image and consequent preoccupation with weight control displayed by women in today's society.

The issue of weight control may be an even greater concern amongst female athletes. The dictate of coaches and the desire for exceptional performance often pressure the female athlete to strive for unrealistic weight goals (Calabrese et al. 1983). Aberrant eating behaviors are common in female athletic populations, particularly those participating in activities which equate leanness with success (Borgen and Corbin 1987). Calorie restriction and removal of fat sources from the diet are strategies often used by these women in an effort to control weight (Loucks 1988). Vegetarianism is also common (Slavin 1984).

A consequence of combining poor nutritional practices with a rigorous training program may be marked suppression of the hypothalamic-pituitary-ovarian (HPO) axis. A high frequency of menstrual dysfunction has been reported in female endurance athletes, particularly those participating in weight-bearing activities (Fichter & Pirke 1984). Athletic amenorrhea or the absence of menstruation in association with high intensity physical activity represents the extreme manifestation of the disorder. Changes in endocrine function demonstrated by these women include reduced circulating levels of estrogenic and gonadotropic hormones and altered luteinizing hormone (LH) secretory patterns (Nelson et al. 1986, Loucks et al. 1989). Chronic elevation of cortisol is commonly observed (Villaneuva et al. 1986, Ding et al. 1988). Poor nutritional habits have been implicated as possible contributing factors (Nelson et al. 1986). Anorexia nervosa is an eating disorder characterized by self-inflicted starvation resulting in severe weight loss (Wilson & Eldredge 1992). Similar hormonal aberrations have been demonstrated by anorexic individuals (Boyar et al. 1974, Boyar et al. 1977, Gold et al. 1986). As well, reproductive failure has been reported in animal populations confronted with food shortages (Foster & Olster 1985, Bronson & Heideman 1990).

The hypothalamic-pituitary adrenal (HPA) axis, which is activated in response to a physiological stressor, has been cited as a possible locus for the underlying pathophysiology of reproductive dysfunction in both anorexia nervosa and athletic amenorrhea (Gold et al. 1986, Loucks et al. 1989). A high number of corticotropin releasing hormone (CRH) receptors exist in close proximity to gonadotropin releasing hormone (GnRH) neurons within the median eminence (De Souza et al. 1984). Possible synaptic contact between CRH and GnRH neurons may explain the mechanistic link between these two axes. High levels of CRH have been shown to suppress GnRH release (Gambacciani et al. 1986).

Statement of the Problem

Previous research has resulted in the following observations upon which this study is based:

1. A pursuit of thinness is prevalent amongst women in today's society (both athletic and non-athletic populations).
2. Calorie restriction and removal of fat sources from the diet are popular strategies used by women to control weight.
3. The incidence of menstrual irregularities is higher in female athletes in comparison to sedentary populations.
4. A link may exist between nutritional status and reproductive function.
5. Reproductive hormone changes which include reduced levels of gonadal steroids and altered gonadotropin secretory patterns are evident in both athletic amenorrhea and anorexia nervosa. Serum levels of cortisol tend to be chronically elevated in both syndromes. Vegetarian diets which tend to be low in fat have been associated with similar changes.
6. The adrenal axis may be capable of eliciting changes in the reproductive axis. Close proximity of CRH and GnRH neurons within the median eminence may allow synaptic contact and a possible means by which CRH can modulate the HP0 axis.

Justification for the Study

The importance of examining the endocrine changes exhibited by these women cannot be understated. Hypoestrogenism resulting in amenorrhea has been associated with premature bone loss (Marcus et al. 1985, Fisher et al. 1986, Drinkwater et al. 1986). Pathological skeletal conditions such as stress fractures and scoliosis have been noted in young hypogonadic athletes (Marcus et al. 1985, Warren et al. 1986). The reversability of these osteoporotic changes may be incomplete (Drinkwater et al. 1986). As well, young women may be forfeiting the natural protective effects of estrogen against cardiovascular disease. Amenorrheic athletes demonstrate elevated serum total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) levels (Friday et al. 1993). This study noted no difference in apolipoprotein A-1, the precursor to high density lipoproteins in amenorrheic athletes. However another study found the apolipoprotein A-1 levels to be reduced in this group (Lamon-Fava et al. 1989). A protective role against development of coronary heart disease has been attributed to HDL-cholesterols whereas elevated plasma LDL-cholesterol levels are considered a risk factor in its development (Schaefer et al. 1994).

Why certain individuals are more susceptible to athletic amenorrhea has yet to be ascertained. The condition presents as a complex interplay of physiological mechanisms with no one factor being singled out as the primary cause. However, recent evidence suggests amenorrheic women may differ significantly in regards to nutritional status (Deuster et al. 1986, Nelson et al. 1986). Thus, it is important that current research explore extensively the underlying etiology of this phenomenon.

Study Objectives

1. To determine if short-term reductions in dietary fat intake are associated with an alteration in LH pulsatility. Blood samples will be collected every 10 minutes for an 8 hour period during the midfollicular phase (MFP) of two consecutive menstrual cycles and the LH pulsatile pattern measured. The first menstrual cycle of the investigation, during which subjects will consume their usual diet, will serve as a baseline measure. Blood collection in the subsequent cycle will follow a five-day, fat restricted diet.

2. To determine if short-term reductions in dietary fat are associated with changes in serum cortisol concentrations. Blood collected for 8 hours as described above will be analyzed for cortisol. Mean cortisol concentration and area-under-the-curve will be compared.

3. To determine if short-term reductions in dietary fat are associated with changes in luteal phase salivary progesterone profiles. During both the baseline cycle and the diet intervention cycle, saliva will be collected daily from day 12 of the menstrual cycle until onset of menstruation and analyzed for progesterone. Mean progesterone concentration and area-under-the-curve will be compared.

Independent and Dependent Variables

The researcher proposes to examine the acute hormonal changes associated with a short-term dietary fat intervention.

Independent Variable

1. A 5-day, 10% fat diet commencing on day 1-2 of the second menstrual cycle of investigation.

Dependent Variables

1. MFP LH secretory patterns occurring over an 8 hour period
2. MFP serum cortisol concentrations occurring over an 8 hour period
3. Luteal phase salivary progesterone profile

Statistical Hypothesis

Ho : No differences in MFP serum cortisol concentrations, LH pulsatility and luteal phase salivary progesterone profiles will be noted in young eumenorrheic women when subjected to a 5-day fat restricted diet ($M_1 = M_2$).

Ha : MFP serum cortisol concentrations, LH pulsatility and luteal phase salivary progesterone profiles will be different in young eumenorrheic women when subjected to a 5-day fat restricted diet ($M_1 \neq M_2$).

M_1 = Baseline menstrual cycle

M_2 = Diet intervention menstrual cycle

Level of significance is set *a priori* at alpha = $0.05_{2\ \text{tail}}$.

Definition of Terms

1. Human Menstrual Cycle

Normal cycle length has been defined as a range of 25 to 36 days with an average length of 28.7 days (Landgren et al. 1980). Cycle length is calculated from Day 1 of menstrual onset (included) until Day 1 of the subsequent menses .

The ovarian cycle consists of 2 phases:

1. Follicular phase which extends from Day 1 of menstrual onset until the occurrence of the midcycle LH surge. The follicular phase lasts an average of 15.1 days (Landgren et al. 1980). Early follicular phase (EFP) is defined as days 1 through 4 of the menstrual cycle. Mid-follicular phase (MFP) is defined as days 5 through 9 and late follicular phase(LFP) as days 10 through 15 (Backstrom et al. 1982).

2. Luteal phase is defined as that period extending from Day 1, post-LH surge until the onset of the next menstruation (excluded). The length of the

luteal phase is relatively constant and lasts an average of 13 days (Landgren et al. 1980).

LH Profile of the Normal Menstrual Cycle

Modulated by the ovarian steroid feedback system, serum gonadotropin levels and pulse patterns fluctuate throughout the menstrual cycle (Jaffe & Monroe 1980). LH levels increase gradually during the early to mid-follicular phase, culminating in a midcycle surge which induces ovulation. Levels fall steadily throughout the luteal phase until just prior to menses, when levels again begin to rise (Jaffe & Monroe 1980).

Gonadotropin release occurs in episodic fashion in response to GnRH (Dierschke et al. 1970). Pulse frequency increases significantly as the follicular phase progresses (from 1 pulse every 80 mins in early follicular phase to 1 pulse every 53 minutes in late follicular). Pulse amplitude tends to increase as the follicular phase progesses (from 0.43 mIU/ ml per min in early follicular to 0.70 mIU/ml per min in late follicular) (Yen 1991). Pulse frequency tends to decrease dramatically during the mid-luteal phase (1 pulse every 177 minute) with wide variations in pulse amplitude displayed (Yen 1991).

Progesterone Profile of the Normal Menstrual Cycle

In ovulatory cycles, a parabolic progesterone profile is typically seen. Serum levels are low throughout the early to mid follicular phase with a small rise occuring in conjunction with the mid-cycle LH peak. A sharp elevation in progesterone levels occurs approximately 3 days post ovulation. Levels tend to plateau from approximately day 5 to day 10 of the luteal phase before declining rapidly to early follicular levels (Abraham et al. 1972, Landgren et al. 1980).

A mid-luteal serum progesterone concentration exceeding 3.0 ng/ml (9.54 nmol/L) is considered an indication that ovulation has occurred (Israel et al. 1972). However, other investigators advocate the summation of 3 separate luteal phase progesterone values to establish the occurence of ovulation (sum total ≥ 15 ng/ml indicates ovulation) (Abraham et al. 1974).

Saliva has been identified as a convenient and reliable sampling medium for the estimation of plasma steroids (Riad-Fahmy et al. 1983). Steroid concentrations within the saliva reflect the non-protein bound fraction in the blood considered to be biologically active (Riad-Fahmy et al. 1983). Salivary progesterone concentrations have demonstrated significant correlations to serum concentrations (Choe et al. 1983, Wang & Knyba 1985). Normal saliva concentrations of progesterone during the luteal phase of the menstrual cycle range from 100-200 pg/ml or 300-600 pmol/L (Choe et al. 1983, Zorn et al. 1984, Wang & Knyba 1985, Vuorento et al. 1989).

Eumenorrhea

Eumenorrhea, for the purposes of this study, will be defined as the occurrence of 10 or more menstrual periods within the last year.

Amenorrhea

Amenorrhea will be defined as the total absence of menstruation for more than 6 months (Goldfien & Monroe 1994).

Oligomenorrhea

Oligomenorrhea is defined as the occurrence of infrequent menstrual periods with a cycle length exceeding 35 days (Goldfien & Monroe 1994).

2. Hypothalamic-Pituitary-Ovarian (HPO) Axis

Homeostasis of the reproductive system involves a number of chemical messengers derived from either hypothalamic, pituitary or ovarian sources. The hypothalamus which maintains synaptic contact with higher brain centers, has ultimate control over the axis (Knobil 1980). Through the release of hypophysiotropic hormones, the hypothalamus regulates pituitary function which in turn regulates steroidal release from the peripheral endocrine organ, the ovary (Knobil 1980).

Estradiol has been shown to exert a paradoxical negative and positive feedback effect upon gonadotropin release (Knobil 1980, Jaffe & Monroe 1980). During the early follicular phase, when estradiol levels are low, an inhibitory effect (negative feedback) upon gonadotropin release is exerted. However, as steroid concentrations progressively increase, feedback becomes positive, stimulating gonadotropin release from the pituitary. The literature suggests that the 2 steroid hormones, progesterone and estrogen, work in synergy to produce the mid-cycle LH surge (Chang & Jaffe 1978).

Gonadotropin-releasing hormone (GnRH)

GnRH is the hypophysiotropic hormone governing reproductive function. It is produced and stored within neurosecretory cells which originate primarily within the arcuate nucleus of the hypothalamus and terminate primarily in the median eminence (Halasz et al. 1989). Transport occurs through the hypophyseal portal system to the anterior pituitary where it binds to specific GnRH binding sites located on pituitary plasma membrane (Marian et al. 1981). Its function is to regulate gonadotropic (FSH and LH) secretion.

GnRH has been referred to as the "hypothalamic pulse generator" because its pulsatile release (ultradian rhythm) is followed by a corresponding episodic release of gonadotropins (Crowley et al. 1985). The periodicity of the GnRH pulse is approximately 60 to 100 minutes in the adult human (Rasmussen et al. 1989).

Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH)

Luteinizing hormone and its counterpart, follicle stimulating hormone (FSH) are gonadotropic hormones released by the anterior pituitary gland in response to GnRH (Dierschke et al. 1970, Carmel et al. 1977). The gonadotropins bind to specific surface membrane receptors located within the ovary. LH acts primarily upon the thecal cells within the ovary, promoting androgen biosynthesis (Bogvich & Richards 1982). The midcycle surge of LH stimulates ovulation and consequent formation and maintenance of the corpus luteum. FSH exerts its action upon the granulosa cells of the ovary, stimulating aromatization and estrogen biosynthesis and promoting follicular growth and maturation (Catt & Dufau 1991).

Ovarian Steroids

Steroid hormones function as biological regulators and are present in nearly all animal tissues (O'Malley & Strott 1991). The group includes male and female sex hormones, the adrenocortical hormones, the D vitamins and bile acids, all of which are derived from the same intermediate, cholesterol.

Estrogen

The primary function of ovarian estrogens pertains to the female reproductive processes (Steinetz 1983). The estrogens are responsible for growth and development of the sexual organs and breasts. Functions include pubertal development of the secondary sex characteristics, female fat distribution and maintenance of female libido and sexual behavior. Estradiol is responsible for ovum maturation and in concert with progesterone, causes the cyclical decidualization of the endometrium. It acts directly on the pituitary gland, affecting gonadotropin secretion through positive and negative feedback mechanisms.

As well, estrogen functions in several important non-reproductive capacities. Skeletally, it stimulates long-bone growth and epiphyseal closure during puberty (Krabbe et al. 1979). Estrogen aids in the prevention of osteopenia by counteracting the effect of parathyroid hormone and reducing the bone resorptive process (Atkins et al. 1972). Estrogen protects the cardiovascular system by increasing serum levels of high-density lipoproteins (HDL), decreasing levels of low density lipoproteins and reducing blood cholesterol levels (Schaefer et al. 1983, Jensen et al. 1987).

Progesterone

Progesterone functions primarily in preparation of the reproductive tract for possible blastocyst implantation and maintenance of the appropriate environment throughout pregnancy (Hadley 1988). This includes enhancement of endometrial decidualization and stimulation of breast glandular development.

3. Hypothalamic-Pituitary-Adrenal (HPA) Axis

The hypothalamic hormone, corticotropin-releasing hormone (CRH) has been recognized as the prime mediator of pituitary-adrenal function, regulating the biosynthesis and secretion of adrenocorticotrophin hormone (ACTH) from the anterior pituitary gland (Taylor & Fishman 1988). Together, the hypothalamus and pituitary form an interface controlling the secretion of glucocorticoids and mineralocorticoids from the adrenal cortex.

Cortisol

Cortisol is a steroid hormone produced and secreted from the zona fasciculata region of the adrenal cortex in response to stimulation from ACTH (Tyrrell 1994).

Physiological Actions of Cortisol

Cortisol is known as a glucocorticoid because of its profound ability to affect glucose metabolism. This effect is primarily anabolic within the liver where it promotes gluconeogenesis (glucose biosynthesis) but catabolic within muscle and adipose tissue (Coufalik & Monder 1981). Cortisol prevents glucose uptake by these peripheral tissues (Tomas et al. 1979). It stimulates lypolysis within adipose and increases lactate release from muscle (Tomas et al. 1979). Through these actions, free fatty acids and amino acids are made available as substrates for gluconeogenesis within the liver. Elevated cortisol levels effectively antagonize the actions of insulin and occur in response to low blood glucose levels such as occur during states of starvation (Tomas et al. 1979).

Glucocorticoids perform a number of other important physiological functions. By decreasing leukocyte production and circulatory migration to an injury site, they effectively reduce the inflammatory response (Craddock 1978). Glucocorticoids are released in response to stress, enhancing the actions of catecholamines (Munck et al. 1984). Thus, cardiac output, peripheral vascular resistance and free fatty acid release from the lipocytes are all stimulated in stressful states. Glucocorticoids have a catabolic effect upon bone tissue because they enhance the bone resorption process while reducing bone accretion (Baylink 1983). They affect calcium metabolism by reducing its absorption from the intestine and increasing excretion through the kidney (Hahn et al. 1981).

Glucocorticoids are responsible for the feedback control of both ACTH and CRH (Keller-Wood & Dallman 1984).

Cortisol Secretory Patterns

Cortisol has a circadian pattern of release with wide fluctuations in serum concentrations occurring within a 24-hour period coincidental with the wakesleep schedule (Weitzman et al. 1970, Krieger 1975). Levels tend to be lowest in the evening, prior to onset of sleep. Secretion occurs in abrupt episodes beginning sometime after the third hour of sleep. Concentrations rise throughout the sleep, peaking approximately one hour after rising. Levels fall throughout the day with the exception of several secretory episodes which occur roughly in conjunction with normally scheduled mealtimes. Cortisol release is controlled by the circadian periodicity and episodic secretion of ACTH (Krieger 1975).

HPO - HPA Link

Stress-induced release of CRH may be capable of modulating GnRH release from the hypothalamus. CRH receptors are located in close proximity to GnRH neurons within the median eminence (DeSouza et al. 1984). GnRH release has been shown to decrease in the presence of CRH (Gambacciani et al. 1986).

Study Delimitations and Limitations

Study Delimitations

1. Subjects consisted of ten non-athletic, eumenorrheic women between the ages of 20-23 consuming a normal mixed diet. Exclusion criteria included a recent weight loss of greater than 10% and extreme thinness (BMI < 18) or obesity (BMI > 30). Repeated measures study design was developed with each subject serving as her own control.

Study Limitations

1. The study was limited to females between the ages of 20-23, restricting relevance to this sex and age group only.
2. A small subject population (n=10) was recruited. However, subjects served as their own control thereby increasing statistical power.

3. Limited information is available regarding the effect of dietary macronutrients on endocrine function. The manipulation of dietary fat in this experiment was extreme but of short duration and it was unknown whether this intervention would be sufficient to elicit a significant effect.

4. Average fat intake normally consumed by subjects was expected to approximate 30%. However, individual differences in average daily fat intake meant a greater reduction in fat for some subjects in comparison to others.

5. The reliability of the 3-day dietary record as a research tool has been questioned by some investigators due to the tendency of subjects to under or overestimate actual daily intake.

6. In order to reduce dietary fat with caloric intake remaining constant, manipulation of other dietary macronutrients becomes necessary. For this study, the decrease in dietary fat was associated with an increase in carbohydrate content to approximately 75% of caloric intake. The increase in complex carbohydrates resulted in the inadvertent but inevitable manipulation of dietary fibre; a dietary component which has been implicated as a possible factor in menstrual cycle aberrations. The possibility exists that other dietary nutrients may also have been manipulated and were directly or indirectly responsible for any occurring hormonal changes.

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CHAPTER TWO

LITERATURE REVIEW

PART 1

Introduction

A high incidence of menstrual dysfunction has been noted amongst highly trained endurance athletes participating in weight-bearing activities (Nelson et al. 1986, Warren 1990, De Souza & Metzger 1991). Investigators have reported that up to 50% of competitive women runners are amenorrheic (Fisher et al. 1986) compared to 2-5% of the normal population (Carlberg et al. 1983). Furthermore, a high incidence of delayed menarche has been reported in those athletes who begin training at an early age (Frisch et al.1980, Warren et al. 1986).

Further investigations into this problem have uncovered menstrual disturbances in highly active menstruating women. These include defective LH release patterns (Cumming et al. 1985, Fisher et al. 1986), anovulatory cycles (Bullen et al. 1985) and luteal phase suppression (Shangold et al. 1979, Bullen et al. 1985). Comparisons between highly trained long-distance runners, recreational joggers and sedentary women have demonstrated significant differences in menstrual function between these three groups suggesting that severity of menstrual dysfunction may be directly proportional to exercise intensity (Dale et al. 1979). Unfortunately, these changes are inconsistent in women training at similar intensities. It is possible that with intense exercise, a continuum of hormonal changes exists with amenorrhea being the extreme manifestation to which only susceptible individuals succumb. Dale et al. (1979) suggest that an oligo-amenorrhea threshold, dependent upon a number of contributing factors, exists for each woman.

Intensity of training (Marcus et al. 1985), low percentage of body fat (Frisch & McArthur 1974), stress (Schweiger et al. 1988) and poor nutritional habits (Warren 1990) have all been cited as etiologic factors in this phenomenon. As yet, researchers have been unable to unequivocably distinguish a prime factor in the development of athletic amenorrhea. This fact has lead investigators to suggest that the condition is multifactorial in development. However, conflicting results could also be a reflection of the wide variety of research methodologies utilized by investigators in the field. Problems associated with this type of research include:

1. Universal definitions of normal menstrual function, including the terms amenorrhea and oligomenorrhea, have yet to be established. Normalcy, in terms of menstrual cycle length and frequency varies widely amongst researchers.
2. A wide variety of research methods have been utilized to establish menstrual integrity. These include: subjective evaluation of menstrual function through the use of questionnaires, basal temperature recording relying heavily on subject compliance, and a multitude of techniques to evaluate hormonal status, including blood, urine or saliva sampling. The difference in protocols for these hormonal evaluations often makes comparisons difficult. Results from many investigations may not adequately reflect the normal circhoral and diurnal rhythms associated with hormonal release. A single morning serum cortisol sample may not be an accurate representation of HPA function. Infrequent sampling of LH may result in missed pulses and a misrepresentation of true LH pulsatility. Steroidal and gonadotropin concentrations fluctuate widely according to phase of the menstrual cycle making it impossible to compare values obtained during different phases.

3. Maturity of the reproductive system confounds results in investigations which compare teenaged athletes to older controls. Approximately one-third of all menstrual cycles in girls ages 14-20 years may be anovulatory (Vuorento & Huhtaniemi 1992). Gynecological age, in terms of postmenarcheal years, is an important determinant of system maturity. Thus, it becomes difficult to determine if results observed in the teenaged girl are a consequence of the imposed intervention or an immature reproductive system.

4. Levels of training and types of activity compared tend to be inconsistent. Runners are frequently the subject group of choice with level of training being defined in terms of distance accomplished per week. It is not uncommon to see high mileage defined as > 25 miles/week in one study and > 45 miles/week in another. Also, girls training at intense levels prior to menarche tend to experience onset of menstruation at a later age than those who are sedentary (Malina et al. 1978). Thus, it is important to establish duration and intensity of training prior to menarche, when considering individuals as possible candidates for investigation.

5. Length of diet interventions and degree of dietary variable manipulation vary widely. Manipulation of one dietary variable may result in the inadvertent manipulation of another, making it difficult to pinpoint the causal element.

6. Diet records which are used to measure dietary intake are subject to recording errors. The tendency to over- or underestimate food intake is a problem, particulary when dealing with a group already prone to eating disorders. The 3-day diet record shows good to strong agreement with the 7-day dietary record for estimates of calories, protein, fat and carbohydrate ($r = 0.79, 0.76, 0.74, 0.90$, $p < 0.005$ respectively) and should be considered an adequate estimate of diet quality (Stuff et al. 1983). Extending the time over

which records are collected in an effort to increase accuracy, interferes with subjects' lifestyles, causing irritation and a consequent erosion of reliability.

When differences in the literature have finally been reconciled, a true understanding of the underlying etiology of athletic amenorrhea will hopefully ensue. The following discussion will examine the related factors associated with this phenomenon.

Menstrual Cycle Changes Associated With Exercise

Menarcheal Delay

Athletic females tend to experience the onset of menstruation at a later age than sedentary women. A study by Malina et al. (1978) compared the menarcheal age of 110 non-athletic women to 130 athletes, grouped according to sport and level of competition. The athletes experienced menarche at a significantly later age than the non-athletes. As well, Olympic calibre volleyball players achieved menarche at a significantly later age than all groups of athletes participating at the high school and college level except those involved in gymnastics, track and tennis.

Similarly, highly trained ballet dancers experience menarche at a significantly later age than age-matched sedentary controls (Warren 1980). This study observed that pubertal progression was directly related to exercise intensity with rapid gains occurring during periods of inactivity. Dancers experienced menstrual onset during periods of forced rest only to undergo recurrence of amenorrhea upon resumption of exercise. Warren suggests that exercise intensity in combination with dietary restrictions present an energy drain to the body, leaving it incapable of sustaining the metabolic demands of reproduction. Thus, reproductive/ovarian function, an event heralded by pubarche and menarche, is delayed until the energy drain desists.

Secondary Amenorrhea and Oligomenorrhea

The frequency of secondary amenorrhea (absence of menstruation following menarche), and oligomenorrhea (irregular menses) in athletic populations is uncertain owing in part to lack of universal definitions for these conditions in conjunction with a wide variety of study methodologies (Arendt 1993). A study of 237 runners conducted by Sanborne et al. (1982) reported that more than 25% of competitive female runners experience less than 3 menstrual periods a year. Carlberg et al. (1983) suggest the prevalence of oligo-amenorrhea may be as high as 50% in this group. A cross-sectional survey of 168 women by Dale et al. (1979) found the frequency of oligomenorrhea and amenorrhea increased as mileage increased. In comparing 3 groups of women, those running in excess of 30 miles per week, those running between 5 and 30 miles per week and a group of non-runners, the frequency of menstrual irregularities was 34%, 23% and 4%, respectively.

However, the association between volume of training and development of secondary amenorrhea has been inconsistent. Participation of 14 normally menstruating women in a 15-month marathon training program failed to produce one incidence of amenorrhea despite reaching a final average weekly mileage exceeding 50 miles (Boyden et al. 1982). However, 13 of the 14 women reported menstrual changes including decreased menstrual flow, increased intercycle length and intermenstrual bleeding. In a survey by Shangold & Levine (1982) which profiled 394 female marathon participants, the frequency of oligomenorrhea or amenorrhea was 24% throughout the training season. However, 91% of those women who were regularly menstruating prior to training continued to menstruate normally throughout training. Irregular menses was more likely in those women who were irregular or amenorrheic prior to training. The fact that certain women are more susceptible than others to volume of training suggests that another factor predisposes these women to development of menstrual irregularities. However, dietary factors were not examined in this particular study.

Anovulation and Shortened Luteal Phase

The luteal phase defect has been defined as diminished progesterone production as the result of inadequate corpus luteum development (Jones 1976). Cycles in which a shortened luteal phase occur are characterized by reduced follicular FSH and estradiol levels and decreased luteal phase progesterone secretion, all of which suggest a defect in follicular maturation (Sherman & Korenman 1973). The incidence of anovulation and shortened luteal phase in the general college-aged population has been estimated at 10.6 and 15.8% respectively (Bullen et al. 1985).

Diminished luteal function has been documented in menstruating well-trained athletes (Loucks et al. 1989) and recreational runners (Broocks et al. 1990). During a study of 28 eumenorrheic women subjected to an intense 8-week exercise program, 43% experienced anovulatory cycles while 38% exhibited a shortened luteal phase (Bullen et al. 1985). Severity of luteal phase abnormalities increased in those women who were subjected to a weight-loss program during the training period. Shangold et al. (1979) followed an individual athlete through 18 consecutive menstrual cycles. The luteal phase during low mileage months (< 5 miles/week) lasted approximately 13 to 14 days. This length was dramatically reduced to under 9 days when mileage exceeded 35 miles per week. Analysis of low mileage and high mileage months detected no significant change in luteinizing hormone levels. However, reduced progesterone levels indicative of suppressed luteal phase function were evident during active cycles.

Changes in Hormonal Profiles

Decreased serum estradiol (Dale et al. 1979, Marcus et al. 1985, Fisher et al. 1986, Drinkwater et al. 1990) and progesterone levels (Dale et al. 1979, Drinkwater et al. 1990) have been well documented in female endurance athletes. Amenorrheic women display decreased LH pulse frequency and erratic pulse behavior (Veldhuis et al. 1985, Loucks et al. 1989). Loucks et al. (1989) reported that amenorrheic athletes displayed reduced LH frequency in comparison to eumenorrheic counterparts who in turn, display reduced LH frequency in comparison to sedentary controls. This is in agreement with observations by other investigators who have noted changes in hormonal function occuring in conjunction with exercise without obvious changes in menstrual status. Defects in LH pulsatility, including decreased pulse amplitude and frequency, have previously been observed in athletes despite the presence of apparently normal menstrual periods (Cumming et al. 1985). Thus, with training, a continuum of hormonal responses may exist. The factor which triggers further progression along the continuum to the amenorrheic state is unknown.

Mechanisms by which the Menstrual Cycle is Changed

Impaired Gondadotropin Response

The mechanism underlying exercise-associated reproductive dysfunction has been the focus of much research. In order to assess the effects of exercise on pituitary function, Boyden et al. (1984) placed normally menstruating women on a rigorous running program. The gonadotropin response to exogenous GnRH progressively decreased with the gradual increase in mileage. Despite the rigorous training program, no participants in this particular study became amenorrheic. However, the majority of women reported menstrual changes during the training period. Boyden et al. (1984) suggests an impaired gonadotropin response may be a result of reduced pituitary gonadotropin reserves. According to these authors, lack of correlation between gonadotropin responses and estradiol levels precludes the possibility that feedback sensitivity was altered as a result of exercise.

These results conflict with those from other studies. An exaggerated gonadotropin response to exogenous GnRH administration has been demonstrated in amenorrheic athletes in comparison to sedentary controls (Reame et al. 1985, Veldhuis et al. 1985, Loucks et al. 1989). Loucks et al. (1989) noted the gonadotropin response in cyclic athletes was less than amenorrheic athletes but greater than that demonstrated by sedentary controls. Exaggerated response to GnRH administration indicates accumulation of LH pools within the pituitary; a direct result of reduced LH pulse frequency. The fact that the pituitary gland remains responsive to exogenous GnRH suggests disruption of the system is occurring at a higher level in the axis; most likely with the pulse generator release of GnRH.

Involvement of the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Exercise represents a physiological stressor whereby a variety of hormonal responses are stimulated. The adrenal axis, which is activated in response to stress, has been linked to gonadal axis suppression (Rivier et al. 1986). Long term glucocorticoid treatment has been associated with menstrual disorders (Sakakura et al. 1975). This study found the LH response to synthetic GnRH injection was significantly depressed in women undergoing prolonged (> 1.5 months) prednisolone treatment. Furthermore, menstruation was induced in amenorrheic women receiving prednisolone simply through the injection of exogenous GnRH suggesting that the main site of glucocorticoid action occurred at the hypothalamic level. In the primate, inhibition of gonadotropin release in response to exogenous CRH administration occurs in the absence of the adrenal glands (Xiao et al. 1989) This contradicts the previous study (Sakakura et al. 1975) and suggests that gonadotropin release is mediated by central mechanisms rather than directly by glucocorticoid excess.

It has been postulated that corticotropin-releasing hormone (CRH) is capable of modulating GnRH release from the medial basal hypothalamus. The CRH pathway which regulates secretion of the pro-opiomelanocortin peptides, including ACTH and ß-endorphin, terminates in the median eminence (Palkovitz 1977). CRH receptors have been identified in high concentrations in this area (De Souza et al. 1984). As well, an extensive plexus of GnRH neurons terminates in the median eminence (Halasz et al. 1989). Exposure of GnRH terminals to high concentrations of CRH has been shown to suppress GnRH release. *In vitro* examinations of rat mediobasal hypothalami and isolated median eminences demonstrate a dose-related reduction in GnRH release when incubated in increasing concentrations of CRH; a response which is effectively blocked by a CRH receptor antagonist (Gambacciani et al. 1986). Reduced LH responsiveness to CRH injection has been documented in live rats (Petraglia et al. 1987). As well, pulsatile release of LH is inhibited in rats exposed to the stress of repeated electric shocks; a response which is effectively blocked with the administration of a CRH antagonist (Rivier et al. 1986).

Endogenous opiates may be the mechanism by which CRH inhibits GnRH release. ß-endorphin is released into the medium eminence in large concentrations in response to CRH secretion (Krieger et al. 1980). Suppression of GnRH pulsatile release is suppressed by endogenous opioids; an action which is attenuated in the presence of an opiate-receptor blockade (Van Vugt et al. 1984, Petraglia et al. 1989).

Acute Adrenal Axis Response to Exercise

Acute bouts of strenuous exercise have been associated with an accompanying rise in serum cortisol and ACTH levels (Baker et al. 1982, de Meirleir et al. 1986, Luger et al. 1987). Exercise intensity appears to play an important role. Luger et al. (1987) noted a rise in serum ACTH and cortisol in direct proportion to exercise workload. Kraemer et al. (1989) report that blood lactate levels are significantly correlated with ACTH and cortisol levels. Activation of the adrenal axis has been shown to occur only when blood lactate concentrations exceed 4 mmol/liter (de Meirleir et al. 1986).

Physical conditioning may or may not alter the cortisol response to exercise. Chronically exercised rats display significantly smaller increases in cortisol and ACTH during bouts of acute exercise when compared to sedentary rats (Watanabe et al. 1991). The conditioned rats displayed a blunted ACTH response to exogenous CRF injection suggestive of a pituitary level adaptation. In human studies, Cumming et al. (1981) were unable to find significant differences between female runners and non-runners in exercise-induced cortisol responses. Luger et al. (1987) also found no difference between trained and untrained subjects at any given exercise intensity, determined as a percentage of VO_2max. However, the cortisol response was closely coupled to plasma lactate and not surprisingly, the trained group required significantly higher absolute workloads in order to reach comparable blood lactate levels. Thus, higher workloads were required to elicit an adrenal axis response in the well-trained individual. Method of training employed by the individual may make a difference. Kraemer et al. (1989) reported that a diminished adrenal axis response to maximal exercise occurred predominantly in those individuals who included a significant anaerobic component to their training program.

Exercise and Resting Cortisol Levels

Chronic hypercortisolism has been well documented in athletic populations. Luger et al. (1987) reported elevated evening levels of both cortisol and ACTH in highly trained males when compared to sedentary counterparts. Elevated resting serum cortisol levels have also been demonstrated in female athletes (Cumming et al. 1981, Loucks et al. 1989) in conjunction with a diminished ACTH response to exogenous CRH administration (Luger et al. 1987, Loucks et al. 1989). In exploring the underlying mechanism of this phenomenon, it is possible that excessive CRH production in response to the stress of exercise causes down-regulation of anterior pituitary CRH receptors effectively decreasing pituitary responsiveness. Watanabe et al. (1991) placed a group of sedentary rats on a 5-week regimen of daily CRH injections. A blunted ACTH response to a CRH bolus was produced, similar to that displayed by chronically exercised rats.

Hohtari et al. (1988) noted no difference in peripheral CRH levels between amenorrheic and eumenorrheic athletes at rest or during an acute exercise test. However, the author admits that peripheral serum CRH concentrations may not be an accurate reflection of hypothalamic CRH content. In a later study, Hohtari et al. (1991) demonstrated differences in the ACTH response to exogenous CRH between eumenorrheic and amenorrheic athletes

A blunted response was noted in the latter group suggesting that changes in the HPA axis may be responsible for changes in menstrual function. Endogenous opiates which are released in response to CRH have also been implicated. Infusion of naloxone (an opiate inhibitor) to highly competitive oligomenorrheic swimmers produced significantly greater LH responses in comparison to eumenorrheic counterparts (Russell et al. 1989).

Athletic Amenorrhea and HPA Function

Hypothalamic-pituitary-adrenal axis function has been compared in amenorrheic and eumenorrheic athletes by a number of researchers. The results from these studies are depicted in Table 2.1.

Villaneuva et al. (1986) reported significantly higher resting cortisol levels in trained groups when compared to matched controls. However, 3 studies noted differences in cortisol levels in relation to menstrual status with amenorrheic athletes demonstrating elevated levels compared to eumenorrheic athletes and controls (Cumming et al. 1981, Loucks et al. 1989, Kanaley et al. 1992). The study by Loucks et al. (1989) may be a more accurate reflection of chronic adrenal hyperactivity because cortisol levels were measured over a 24-hour period as opposed to a single AM sample.

Again, investigations into the underlying mechanism intimate hypothalamic-pituitary involvement. Administration of ACTH to amenorrheic, eumenorrheic athletes and sedentary controls produced similar cortisol responses in all three groups (Villaneuva et al. 1986). Thus, it seems unlikely that changes are a result of diminished adrenal sensitivity. The degree to which reproductive function is disturbed may be dependent upon the extent to which changes have occurred within the HPA axis; a factor which varies between individuals. Loucks et al. (1989) speculates a resetting of the HPA

Table 2.1 Resting cortisol levels and cortisol response to exercise in amenorrheic, eumenorrheic athletes and control group: results from various investigations.

REFERENCE	SUBJECTS	RESTING SERUM CORTISOL	24-HR URINE CORTISOL	CORTISOL RESPONSE TO EXERCISE
	(runners)			bike @ 60rpm til max
Cumming et al. 1981	Am	elevated in runners		elevated
	Eu	compared to	–	elevated
	C	non-runners		elevated, initial decline (no sig diff betw groups)
	(runners)	Late AM sample (ng/ml)		40 mins@80% VO2max
Loucks & Horvath 1984	Am	147 ± 25 (n = 7)	–	72 ± 54 ng/ml
	Eu	106 ± 13 (8)		182 ± 25 ng/ml
	(runners)	AM sample (ng/ml)	µg/24hr	
Villaneuva et al. 1986	Am	118.3 ± 5.9 (12)	45.1 ± 7.2 (6)	–
	Eu	109.6 ± 1.9 (15)	38.1 ± 6.9 (9)	–
	C	71.0 ± 3.9 (10) **	13.9 ± 2.8 (4) **	–
	(endurance)	24 hr mean (nmol/l)	g/24 hr	
Loucks et al. 1989	Am	230 ± 10 (9) **	119.1 ± 9.0 **	–
	Eu	180 ± 10 (9)	90.6 ± 9.8	
	C	170 ± 10 (8)	79.7 ± 4.9	
	(runners)	AM sample (nmol/l)		90 mins @ 60%VO2max
Kanaley et al. 1992	Am	524.2 ± ? (6)	–	-significantly elevated
	Eu	325.5 ± 22 (8) **		-slightly < basal levels

** significantly different from other group or groups.
AM serum norms: 140-552 nmol/L
24-hour urine norms: 20-90 µg/24 hrs.

axis may occur through activation of the negative feedback mechanism which in turn occurs as a result of elevated CRH and cortisol levels. In this study, administration of CRH to amenorrheic athletes, eumenorrheic athletes and sedentary women produced blunted ACTH and cortisol responses only in the active groups (Loucks et al. 1989). While these responses were more impaired in the amenorrheic group in comparison to the cycling athletes, the difference was not significant. All 3 groups displayed similar basal ACTH levels and 24-hour ACTH pulse patterns.

Undernutrition and the HPA Axis

A state of marked hypercortisolism has been documented in cases of protein-calorie malnutrition (Alleyne & Young 1967, Smith et al. 1975) and anorexia nervosa (Warren & Van de Wiele 1973, Gold et al. 1986). The anorexic person presents with normal serum levels of ACTH yet displays reduced plasma ACTH responses to CRH (Gold et al. 1986). Reversal occurs when weight is normalized and maintained. The hormonal profile of anorexia nervosa is markedly similar to that of athletic amenorrhea (Loucks et al. 1986).

As well, starvation and weight loss in normal individuals has been associated with adrenal axis changes. Total starvation of normal weight women for a 3 week period produced elevated 24-hour plasma cortisol levels and a prolonged plasma cortisol half-life (Fichter et al. 1988). Similar deviations have been documented in cases of anorexia nervosa (Boyar et al. 1977, Caspar et al. 1979). The lack of Cushingoid symptoms displayed by patients with anorexia is explained by a reduction in cellular glucocorticoid receptors (Kontula et al. 1982). Elevated levels of CRH (suggestive of hypersecretion) have been noted in the cerebrospinal fluid of anorexic patients (Hotta et al. 1986). A series of studies involving rhesus monkeys performed by Cameron and associates noted reductions in gonadotropin levels coincidental with acute elevations of serum cortisol levels when the animals were subjected to a calorie restricted intake (Cameron 1989).

Conclusion

Intense physical exercise and a state of malnourishment can be considered stressors to the body thereby invoking an adrenal response. It is possible that activation of the hypothalamic-pituitary-adrenal axis may affect normal functioning of the gonadal axis although the exact mechanism whereby these aberrations occur has yet to be pinpointed.

PART 2

Related Factors in the Development of Athletic Amenorrhea

Introduction

The exact factor or combination of factors responsible for the observed menstrual changes have yet to be determined. A number of variables have been implicated in this condition.

Stress of Competition

Athletes performing at the elite level are often highly competitive and goal-oriented. Researchers have questioned if stress of competition is instrumental in the disruption of normal reproductive function. High levels of stress in athletes have been associated with reduced mean luteal phase estradiol and progesterone levels (Schweiger et al. 1988). This study, which surveyed participants from a number of sports with varying training intensities and levels of competition, asked participants to subjectively rate the stress involved in various aspects of life. High stress and low calorie intake were both found to be highly correlated with impairment of luteal phase serum progesterone levels. Schweiger et al. (1988) cautions that neither of these factors should be considered independent variables in this phenomenon, but as interactive modes working in combination with each other and other variables to impair menstrual function.

Other investigators have been unable to establish a relationship between stress and athletic amenorrhea. Athletes have demonstrated normal psychological profiles regardless of menstrual status (Schwartz et al. 1981). Warren (1980) compared the menarcheal age of highly motivated ballet dancers to similarly intense music students. While mean age for onset of menstruation for the music students was similar to the mean value of the general population, the dancers first menstruated at a significantly later age. Both groups were subjected to high levels of stress, yet menarche was delayed only in the dancers making it unlikely that differences were related to stress alone.

A psychological evaluation of amenorrheic and eumenorrheic athletes by Loucks & Horvath (1984) was unable to establish differences in psychological well-being between the two groups. In fact, both groups scored favorably in comparison to the general population in all measured parameters of positive mental health. The authors found no indication that stress was in any way related to reproductive dysfunction.

Body Composition

In the early seventies, a report by Frisch & McArthur (1974) proposed that a miminum amount of stored adipose was required for normal menstrual function; 17% body fat being deemed necessary for the induction of menarche and 22% for the maintenance of normal menses. Athletes participating in certain aesthetic and endurance activities continually strive for unrealistic body fat levels. Some studies report a relationship between adiposity and menstrual dysfunction in athletic populations (Sanborn et al. 1982, Shangold & Levine 1982). Warren (1980) found the incidence of amenorrhea in highly trained dancers to be more prevalent in those of low body fat.

However, the "critical body fat theory" has sustained much criticism and been disputed by a number of investigations which have noted no difference in percent body fat when comparing eumenorrheic to amenorrheic athletes (Marcus et al. 1985, Fisher et al. 1986, Deuster et al. 1986). Cases of normally cycling athletes with a body fat well below the critical fat level have been documented. Marcus et al. (1985) reports one eumenorrheic athlete with a body fat of 4%. As well, amenorrhea has been reported in athletes with a body fat exceeding 22% (McArthur et al. 1980). Pubertal progression has been noted in injured ballet dancers without a change in the lean/fat ratio (Warren 1980).

Nutritional Factors Associated with Athletic Amenorrhea

Reduced Calorie Intake

Stringent dieting is common among athletes continually striving to maintain a competitive edge or meet aesthetic standards. The similarities in endocrine dysfunction which occur between athletic women and the chronically undernourished individual has prompted speculation that aberrant eating patterns contribute to menstrual irregularities. Consequently, athletic diets have been scrutinized by a number of investigators over the past decade.

A summary of several recent publications which have investigated the dietary intake of athletic women is provided in Table 2.2. Comparisons of

TABLE 2.2 Summary of studies investigating nutritional status of athletic groups.

REFERENCE	SUBJECTS	n	INTENSITY km/wk	ENERGY kcal/day	PROTEIN % / intake	FAT %	CHO %
Deuster et al. 1986a	athletes ¶	51	112	2397 ± 104	13	32	55
Deuster et al. 1986b	eu	33	113	2489 ± 132	13	34**	53
	am	12	119	2151 ± 236	13	27	60
Drinkwater et al. 1984	eu	14	40	1965 ± 98.4	16	35	49
	am	14	67	1622 ± 145	19	30	51
Loucks et al. 1989	C (sed /eu)	8	-	1597 ± 212	15	38**	46**
	eu	9	mixed	1804 ± 498	15	27	57
	am	9	mixed	1669 ± 492	14	25	62
Marcus et al. 1985	eu	6	>65	1715 ± 281	21	?	?
	am	11	>65	1272 ± 136	19		
Nelson et al. 1986	eu	17	64	2250 ± 141**	15	34	51
	am	11	56	1730 ± 152	15	33	52
Schweiger et al. 1988	C (sed ¶)	25	-	2101	12	37	46
	athletes ¶	14	mixed	2262	12	38	46
Snead et al. 1992	C (sed /eu)	9	-	1850 ± 160	18	35	47
	eu	19	49	1971 ± 145	19	31	50
	ol/am	13	46	2043 ± 114	17	29	54

** significantly different from other groups ¶ menstrual status undetermined
C = control group eu = eumenorrheic am = amenorrheic ol = oligomenorrheic sed = sedentary

athletic diets to those of sedentary controls have been unable to ascertain significant differences in caloric intake between these 2 groups (Schweiger et al. 1988, Loucks et al. 1989, Snead et al. 1992). However, a number of investigators have noted that while calorie intake is not reduced among athletic women, they do not compensate calorically for their estimated increased energy expenditure. The National Research Council (1980) recommends an intake of 1800-2200 kcal/day (38 kcal/kg/day), for sedentary women between the ages of 23 and 55. This compares favorably with the recommendations of Health and Welfare Canada (1983) who suggest that women between the ages of 19-24 participating in light activity require approximately 2100 kcal/day or 36/kg/day. A nutritional survey of 51 elite women runners by Deuster et al. (1986a) reported the caloric intake in this group to be only marginally higher than the recommended daily intake despite the fact that these women were running an average of 10 miles per day. Deuster suggests that caloric intake should have exceeded 2600 kcal/day or 54 kcal/kg/day in order to maintain a healthy energy balance. Further examination showed that 25% of these women consumed less than the 38/kg/day recommended for sedentary women.

Several studies from Table 2.2 have investigated the relationship between menstrual status and nutritional intake (Drinkwater et al. 1984, Marcus et al. 1985, Nelson et al. 1986, Loucks et al. 1989, Snead et al. 1992). All agree that caloric intake tends to be lower in amenorrheic athletes when compared to eumenorrheics. However, because of high variability in the results, only the study by Nelson et al. (1986) was able to find significant differences in intake between these two groups. Marcus et al. (1985) report the average intake for its amenorrheic group at 1272 kcal/day; an extremely low figure considering volume of running for this group exceeded 65 km per week.

Differences in Macronutrient Consumption

A study by Schwartz et al. (1981) noted a significant reduction in percent of calories consumed as protein in the diets of long distance female runners when compared to recreational runners and sedentary controls. This reduction in protein content was associated with the incidence of amenorrhea. Both the long-distance eumenorrheic runners and the amenorrheic runners consumed more calories than the control group and when this was taken into account, grams of protein consumed by the runners was equivalent to the sedentary women.

Loucks et al. (1989) found fat intake in their athletic group to be considerably less in comparison to sedentary controls; a difference compensated for by a significant increase in carbohydrate intake. Another study which compared the diets of amenorrheic and eumenorrheic athletes found a significant fat reduction in the former group (Deuster et al. 1986b).

Table 2.3 depicts a compilation of data from the studies in Table 2.2 to determine if a particular trend in macronutrient consumption can be detected. When an average of all 8 studies is taken, the athletes consumed 16% of their calories in the form of protein, 31% in fat and 53% in carbohydrate. In general, the athletic diets were lower in fat and higher in carbohydrate than the diets consumed by sedentary controls (15% protein, 37% fat, 46% CHO) .

Table 2.3 Compilation of data from various studies showing mean percentages of macronutrients in athletic diets.

	% Protein	% Fat	% CHO
Controls (sedentary)	15% (3)	37% (3)	46% (3)
range	[12-18]	[35-38]	[46-47]
Athletes *	16% (8)	31% (8)	53% (8)
range	(12-21)	(25-38)	(46-62)
Athletes (eu)	19% (6)	32% (5)	52% (5)
range	[13-21]	[27-35]	[49-57]
Athletes (am)	18% (6)	28% (5)	56% (5)
range	[12-19]	[25-33]	[51-62]

( ) - Number of studies compiled * athletic diets in general, regardless of menstrual status

Furthermore, the athletes' diets differed considerably from the average Canadian diet which is estimated at 15% protein, 38% fat and 47% carbohydrate [Statistics Canada (1986) as reported by Robbins & Robichon-Hunt (1988)]. When menstrual status is taken into consideration, fat intake tends to be lower in amenorrheic athletes when compared to eumenorrheic counterparts (28% vs 32%). Again, this difference is compensated for by an associated increase in carbohydrate consumption.

Incidence of Vegetarianism

A high incidence of vegetarianism has been reported in female athletes (Brooks et al. 1984, Slavin et al. 1984). In comparing groups of amenorrheic and eumenorrheic athletes, Brooks et al. (1984) noted meat consumption to be 5 times greater in the cyclic group. In fact, 83% of the amenorrheic athletes were described as vegetarian as opposed to 13% of the eumenorrheics. While protein intake between the two groups was similar, fat intake was substantially reduced in the amenorrheic group. Similar results were reported by Slavin et al. (1984) in a study of 36 women cyclists. Only 4 of the women studied were consuming a mixed diet. The remaining 32 women were classified as modified vegetarians, totally excluding red meat from the diet. Twelve of the 32 women (38%) were amenorrheic.

Eating Disorders

The differences in diet patterns exhibited by athletes, in addition to their apparent preoccupation with weight management, has promoted the opinion that this group is highly prone to eating disorders (Zucker et al. 1985). However, investigations into this matter do not entirely support this theory (Borgen & Corbin 1987, Snead et al. 1992). Administration of the Eating Disorders Inventory (EDI) (Garner & Olmstead 1984) to a group of runners by Snead et al. (1992) was unable to reveal attitudes and behavioral patterns associated with anorexic tendencies. However, the authors admit that the secretive nature of this anomaly may have hindered data accuracy. This argument seems plausible when one considers the study by Wilmore et al. (1992) in which 6 amenorrheic runners scored within normal range on the EDI despite receiving past treatment for eating disorders.

Nutrition and Reproductive Dysfunction

Nutritional manipulation studies, observations of the dietary and menstrual patterns of vegetarian populations and indepth examinations of the anorexia nervosa patient, have been used as models to understand the relationship between nutrition and reproductive processes.

Calorie Restriction

Reproductive dysfunction in cases of severe calorie restriction has been well documented. Famine conditions in post-World War II Netherlands were likely the cause of delayed menarche experienced by circumpubertal girls exposed to extreme food rationing (van Noord and Kaaks 1991). Bates et al. (1982) describe unexplained menstrual dysfunction in dieting women which was readily reversed upon increased calorie intake and normalization of weight.

An altered hormonal mileau has been recorded in the anorexic individual. Anorexic nervosa is an eating disorder primarily affecting young females and characterized by amenorrhea, extreme weight loss and altered body image (Warren 1973). Endocrine changes associated with this condition include reduced circulating levels of estrogen and gonadotropins (Warren & Van de Wiele 1973). A twenty-four hour LH study by Boyar et al. (1974) noted age-inappropriate (pre-pubertal or early pubertal) LH secretory patterns in 8 out of 9 anorexic women. Feeding and consequent regaining of weight results in the reversal of hormonal aberrations (Warren & Van de Wiele 1973).

In an effort to further investigate the association between calorie restriction and reproduction, experimentation with normally cycling women has been attempted. Institution of a 1000 calorie diet by Pirke et al. (1985) produced luteal phase disturbances in all subjects. Fichter & Pirke (1984) subjected 5 normally menstruating women to a 3-week total starvation period. Within 2 to 3 weeks, infantile patterns of LH secretion were noted in 3 out of 5 women. Menstruation was absent in all subjects throughout the 3 week starvation period and 6 weeks beyond.

Numerous studies involving animal models have been used to examine the effects of undernutrition on reproductive function. Young female rats experienced an abrupt halt to their pubertal development when placed on a calorie restricted diet (Bronson 1986, 1988). Resumption of regular food intake resulted in a rapid developmental "catch-up" reminiscent of the vacationing ballet dancers described by Warren (1980). A decrease in kilocalories is similar to an increase in energy outlay in that both actions create an imbalance in the energy equation. It seems, that when energy needs for everyday metabolic processes including exercise are not being met, processes requiring additional energy such as growth and reproduction, are shut down.

Delayed pubertal development has been restored in food restricted rats solely with the administration of GnRH (Bronson 1986, 1988). Rapid reproductive development occurred despite total absence of growth. The abrupt response to GnRH targets the hypothalamus as the lesion site. Furthermore, food-restricted rats demonstrated severely reduced LH pulsatility. However, LH pulses resumed briefly following the daily food ration suggesting that food intake may regulate LH secretion on an hour to hour basis (Bronson & Manning 1989). In most cases, resumption of normal ad libitum feeding produced normal pulsatility within 24 hours (Bronson 1986, 1988). This same effect has been demonstrated in food restricted cows, where normal LH patterns were reestablished within hours of resuming normal ad libitum feedings (McCann & Hansel 1986).

Effect of Specific Dietary Constituents upon the Reproductive Axis

As previously discussed, differences in macronutrient content of athletic diets have been noted. It has occurred to investigators that certain dietary constituents may contribute to reproductive dysfunction. A study of 59 undergraduate women by Snow et al. (1990) reports that oligomenorrheic women consume less saturated fat and more dietary fiber than eumenorrheic counterparts. Furthermore, a high incidence of menstrual irregularities (Pedersen et al. 1991) as well as a lower incidence of breast cancer (Wynder 1980) in vegetarian populations, has sparked interest in the effect that certain dietary constituents may have upon reproductive hormonal function. Vegetarian diets tend to be lower in protein, saturated fat and cholesterol and higher in fiber content (Pedersen et al. 1991). A recent German study found that early age of menarche, a factor associated with an increased risk of breast cancer, corresponded with high fat consumption by young girls in that country (Merzenich et al. 1993). Lower fat intake often goes hand in hand with increased fiber dietary content due to the increased consumption of complex carbohydrates. Menarcheal age is also highly correlated with fiber intake (Hughes & Jones 1985). An increasing incidence of breast cancer has been associated with affluent societies consuming a high-fat, low-fiber diet (Armstrong & Doll 1975).

The question of whether or not vegetarianism induces menstrual cycle changes has been addressed by Pirke et al. (1986). Placement of eumenorrheic, omnivorous women on a low calorie vegetarian diet for a 6 week period produced a shortening of the menstrual cycle by an average of 5 days. The majority of women became anovulatory during the diet period, displaying significantly reduced serum levels of LH, estrogen and progesterone; responses not evident in a control group consuming a nonvegetarian diet of equal calorie content.

However, the dietary component responsible for these changes remains to be identified. Placement of North American Caucasian women on a vegetarian diet for two months reduced the length of menstrual cycles by an average of 4 days (Hill et al. 1984). Furthermore, these women displayed reduced LH levels and pulse frequency in conjunction with increased LH responsiveness to exogenous GnRH. While calorie content was kept constant with normal intake, the average daily fat content of the diet was reduced from 38% to 33% of total calories with a concommitent rise in complex carbohydrates. In the same study, vegetarian women (South African Negro) were placed on a daily meat ration of 150 grams. The average daily fat intake for these women increased from 30 to 35% of total calories. The average length of the menstrual cycle in these women was increased by 2 days and FSH levels were significantly elevated. Hill further investigated this matter utilizing 2 groups of fertile Black South African women (Hill et al. 1986). One group was subjected to a daily meat supplement while the other was given an isocaloric supplement of soybean. The protein content of both diets was equivalent to the average North American woman's diet. On average, the meat supplemented groups experienced a 3-day increase in menstrual cycle length, increased episodic release of both LH and FSH and reduced gonadotropic response to GnRH administration. The soybean supplemented group experienced no significant changes in hormonal profiles. The addition or removal of animal protein appears to have exerted some effect, perhaps at the level of the hypothalamus, which in turn was manifested by alterations in menstrual function. Factors associated with ingestion of animal protein such as protein quality, increased cholesterol and saturated fat intake may have contributed to these changes.

The relationship between breast cancer and diet has stimulated interest in the effect of low-fat diets on estrogen metabolism. There is suggestion that serum non-protein bound estrogen, but not total estrogen levels, are higher in women with breast cancer (Moore et al. 1982). Low fat diets have been shown to reduce serum non-protein bound estradiol levels (Ingram et al. 1987) and serum estrone sulfate levels (Woods et al. 1989) in women. The latter is an important conjugate of estradiol and estrone and reflects changes in estrogen metabolism.

Indeed, differences in excretory patterns of estrogen have been noted in vegetarian women leading to speculation that dietary factors may be capable of altering estrogen metabolism in some manner (Goldin et al. 1982, Adlercreutz et al. 1986, Lloyd et al. 1991). Goldin et al. (1982) noted increased fecal excretion (2-3-fold) of estrogen in vegetarian women compared to omnivorous women. The former group consumed a significantly lower percentage of fat and significantly more grams of fiber than the latter. Ingestion of animal protein and associated factors (cholesterol and saturated fats) were also significantly reduced. Presumably, increased fecal bulk and higher fecal estrogen levels are related to a higher fiber intake. However, it has also been hypothesized that higher lipid levels within the gut resulting from increased fat intake may enhance reabsorption of biologically active estrogens due to their lipid soluble, low polar properties (Adlercreutz et al. 1986). Thus, those consuming less fat could be experiencing greater loss of estrogen through the feces.

As well, decreased excretion of estrogen metabolites through the urine, indicative of lowered circulating levels of these substances, has been associated with both low-fat (Longcope et al. 1987) and vegetarian diets (Adlercreutz et al. 1986). A study by Lloyd et al. (1991) reported lower concentrations of urinary estrogen in oligomenorrheic vegetarian women in comparison to eumenorrheic vegetarian women. However, no difference was noted between cyclic vegetarian and nonvegetarian women. This study also found significantly greater urinary excretion of LH in the vegetarian group when compared to the non-vegetarian group; a factor which was significantly correlated with total fat intake. However, it should be noted that dietary fiber intake was also significantly increased in the vegetarian group. The fact that low fat in the diet often coincides with increased fiber intake has made it difficult to conclude with certainty which factor (if either) is more relevant as a causal element in this condition.

High concentrations of substances known as phytoestrogens have been identified in the urine of vegetarian women (Adlercreutz et al. 1987). Similar in structure to synthetic estrogens, phytoestrogens are compounds found in fiber-rich vegetables which are capable of exerting mild estrogenic actions within the body. High levels entering the portal circulation may stimulate sex hormone binding globulin (SHBG) production within the liver. An elevation in SHBG (demonstrated by vegetarian populations) would effectively reduce the serum concentration of unbound biologically active estrogen and androgens (Adlercreutz et al. 1987).

Reduced Energy Requirements

The ability of athletic women to maintain normal weight in the face of high training loads and low to average daily calorie intakes has puzzled investigators. It has been suggested that these women conserve energy by effectively reducing their basal metabolic rate (Myerson et al. 1991).

A decline in metabolic rate has been associated with dieting (Bray 1969) and anorexia nervosa (Warren & Van de Wiele1973). It is controversial as to whether or not exercise has the ability to reverse this process. Resting metabolic rate has been shown to decrease in individuals who combine exercise with severe calorie restriction (Phinney 1985, reviewed in Brownell et al. 1987).

It has been suggested by Warren (1983) that menstrual dysfunction in athletic women is a result of huge energy demands in a state of chronic undernourishment. The response resembles that of the !Kung Bushwomen of the Kalahari Desert who experience seasonal anovulation when forced to forage extensively during periods of food scarcity (Van der Walt et al. 1978). Ovarian function is suppressed in Nepali women during periods of heavy fieldwork (Panter-Brick et al. 1993). High caloric expenditure in combination with food shortages place the organism in a chronic state of negative energy balance. Suppression of ovulation may be an adaptive response which the body uses in order to conserve energy for more essential processes.

Conclusion

Reproductive dysfunction in the female athlete presents as a complex intermingling of physiological mechanisms. It is important to understand the underlying etiology of this phenomenon. Adverse consequences of athletic amenorrhea include premature bone loss and possible increased risk of cardiovascular disease. While many factors have been suggested in development of this problem, no one factor can be singled out as the prime cause. However, it is likely that nutritional status plays a major role. Current research appears aimed at determining which aspect of diet of diet provides the signal suppressing reproductive function, the mechanism by which this occurs and why some women are more susceptible than others. The severe repercussions associated with athletic amenorrhea are an important reason why vigorous research must continue in this area.

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CHAPTER THREE

METHODOLOGY

Subjects

Ten (10) females between the ages of 20 and 23 were recruited from the University of Alberta student population to participate in a 3 month study.

Exclusion Criteria

Exclusion criteria included:

1.) cigarette smoking

2.) recent weight loss (> 5 kg)

3.) participation in a regular, intensive training program

4.) use of oral contraceptives or any other medications known to influence menstrual function within the previous 6 months.

5.) vegetarianism or unusual eating habits.

6.) VO_2max exceeding 45 ml/kg/min as measured on a cycle ergometer.

Project Description

The study consisted of 3 phases:

1.) Screening: a series of questionnaires and tests were completed to establish subject eligibility.

2.) Establishment of baseline hormone profile: During the first menstrual cycle, mid-follicular phase measurements of serum cortisol and luteinizing hormone were taken at 10 minute intervals for one 8-hour period. Daily salivary progesterone samples were collected during the luteal phase of the menstrual cycle.

3.) Fat-reduced diet Intervention and hormonal response: Upon onset of the subsequent menstrual cycle, subjects were placed on a 5-day low fat diet. Immediately upon completion of diet, blood and saliva samples were collected as described above.

A schematic diagram of the project is provided (Figure 3.1).

Screening

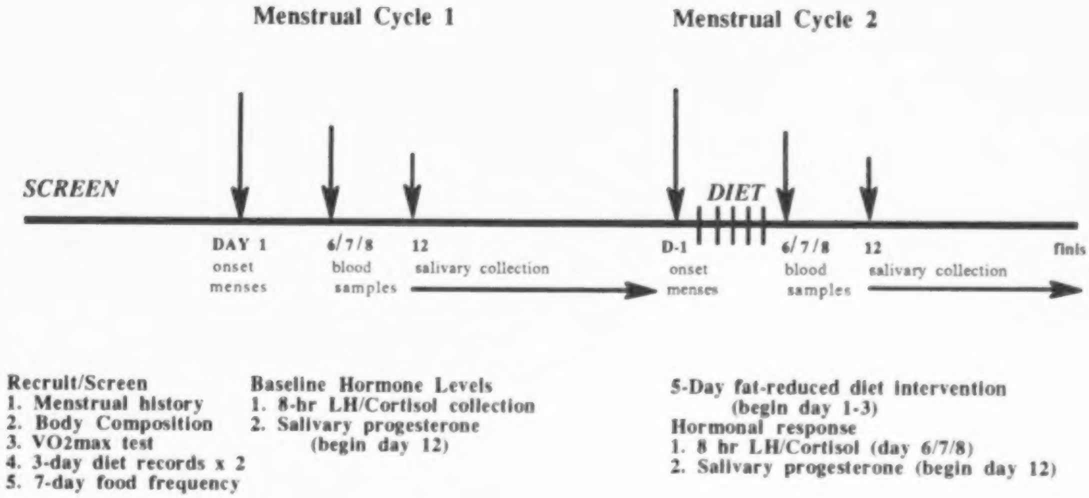
Initial screening included:

1.) **Menstrual history questionnaire**: To establish normal menstrual patterns, each subject completed a short questionnaire describing date of menarche and current menstrual cycle characteristics and symptoms (see Appendix 1).

2.) **Body composition**: Extreme thinness (Boyar et al. 1974) and obesity (Edman & MacDonald 1978) have been associated with changes in hormonal function. Thus, it was necessary to exclude such individuals. Height and weight were recorded and skinfolds (triceps, biceps, subscapular, suprailiac and medial calf) using Lange calipers were measured. Body mass index and sum of 5 skinfolds were calculated as described by the Canadian Standardized Test of Fitness and compared to population norms (CSTF 1986). Subjects scoring over the 85th percentile or under the 15th percentile were excluded from further participation.

3.) **Aerobic fitness assessment**: The purpose was to eliminate subjects with a VO_2max exceeding 45 ml/kg/min which is indicative of a well-trained woman (Wells 1991). Maximal oxygen consumption (VO_2max) was determined using a graded exercise test to exhaustion on a Monark cycle ergometer

Figure 3.1 Schematic diagram of project


Menstrual Cycle 1
Menstrual Cycle 2
SCREEN
DIET
DAY 1
onset
menses
6/7/8
blood
samples
12
salivary collection
D-1
onset
menses
6/7/8
blood
samples
12
salivary collection
finis

Recruit/Screen
1. Menstrual history
2. Body Composition
3. VO2max test
4. 3-day diet records x 2
5. 7-day food frequency

Baseline Hormone Levels
1. 8-hr LH/Cortisol collection
2. Salivary progesterone
(begin day 12)

5-Day fat-reduced diet intervention
(begin day 1-3)

Hormonal response
1. 8 hr LH/Cortisol (day 6/7/8)
2. Salivary progesterone (begin day 12)

(Thoden et al.1982) (see Appendix 2). Respiratory gases were continuously collected and averaged over 20 seconds using the Horizon metabolic measurement system (Sensormedics Inc., Yorba Linda, CA). Heart rate was monitored continuously and recorded each minute using a chest-belt mounted transmitter and watch receiver unit (Polar,™ Port Washington, NY).

4.) **Dietary Intake**: The purpose was to establish normal mixed dietary intake and to ascertain normal dietary intake. Average daily kcal and macronutrient intake, including protein, fat, carbohydrate and fiber content were measured. Each subject completed a 7-day food frequency checklist (Appendix 3) and 2, 3-day dietary intake records. To develop accurate recording skills, each subject received a personal training session. As well, each completed record was reviewed with the individual to eliminate possible misinterpretations by the technician. Records were collected over 2 weekdays and 1 weekend day and both records were collected within a 2 week period. Dietary records were analyzed by a computerized nutrient analysis program (Food Processor™, Esha Research, Portland, Ore).

Reproductive and Stress Hormone Measurements

1.) **Salivary Progesterone Measurements:** Salivary progesterone measurements are a practical and convenient method of monitoring daily levels of this hormone (Chloe et al. 1983). Daily samples were collected from day 12 to onset of menstruation for two consecutive menstrual cycles (Appendix 4). Each morning, prior to eating or brushing the teeth, subjects were instructed to collect a 2 ml sample of unstimulated saliva into the provided containers (Salivet™, Sarstedt, St. Laurent, PQ). This consisted of chewing a cotton wad for a period of 1 minute. Saliva samples were stored in the home freezer until completion of each menstrual cycle upon which it was collected in a cooled container by the researcher. Samples were stored at -80° C until analysis by radioimmunoassay (RIA) technique (see appendix 6).

Serum Luteinizing Hormone and Cortisol Measurements: To determine LH pulse patterns and cortisol levels, blood samples were drawn at 10 minute intervals for an 8-hour period during the mid-follicular phase of both the baseline and fat-reduced diet menstrual cycles (Appendix 5). Subjects reported to the Clinical Investigations Unit, U of A Hospital at 0800 hours on day 6 or 7 of the menstrual cycle. An indwelling intravenous catheter was inserted by a Registered Nurse and sampling commenced following a 60 minute rest period. A 2 ml blood sample was collected every 10 minutes and allowed to coagulate. Samples were centrifuged at 4° C and plasma aliquots stored at -80°C until analysis by RIA.

LH pulse features were analyzed by a computer-assisted program (Monroe 1.1, Zaristow Software, West Morham, Scotland) using the Cluster Analysis Algorithm (Veldhuis & Johnson 1986). Hourly samples were analyzed for cortisol and the area-under-the-curve for the eight hour period was calculated with the aid of a computer program using the "sliding window" technique (Shaw & Foxcroft 1985).

Fat-Reduced Diet Intervention

Upon onset of menstrual cycle #2, (Day 1-2 of the early follicular phase) each subject was placed on a 5-day eucaloric mixed diet consisting of approximately 10% fat, 15% protein and 75% carbohydrate. All meals were prepared by the Clinical Investigations Unit, Nutrition and Food Services at the University of Alberta Hospital. Meals were packed and delivered on a daily basis to the subject to consume at home/school as desired. Subject weight was measured immediately before and after diet period. Example of a diet menu is provided in Appendix 7.

Statistical Analysis

Paired *t* tests (2-tailed) were used to compare data obtained during baseline cycle with that collected during the diet-intervention cycle. Differences were considered statistically different when *P* values were ≤ 0.05. Post-hoc analysis involved the determination of Pearson r correlation coefficients to assess relationships between changes in both endocrine and nutritional variables.

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CHAPTER 4

RESULTS

Subject Characteristics

Subject age ranged from 20-23 years (mean = 21.3 ± 0.4). Aerobic capacity ($\dot{V}O_2$max) on a cycle ergometer ranged from 36-44 ml / kg / min (mean = 38.9 ± 0.98). Height and weight were used to calculate Body Mass Index (BMI). BMI for subjects ranged from 19-28 (mean = 24 ± 0.97). The sum of 5 skinfolds was calculated and compared to Canadian population norms according to methods outlined by CSTF protocol (CSTF 1986). CSTF % for sum of 5 skinfolds ranged from 15-85 % (mean = 36.2 ± 7.6). A summary of subject characteristics is depicted in Table 4.1.

Table 4.1 Subject characteristics

SUBJECT	AGE yrs	VO_2max ml/kg/min	Ht cm	Wt kg	BMI kg/m^2	SOS CSTF %
1	21	36	176.7	83.4	26	15
2	23	36	166.0	60.5	22	25
3	21	42	169.6	57.3	20	85
4	22	42	175.4	67.0	22	65
5	21	44	171.2	73.8	25	45
6	20	36	166.6	72.0	26	40
7	22	37	165.4	69.0	25	15
8	23	39	168.1	76.2	27	22
9	20	41	172.5	83.4	28	15
10	20	36	160.8	50.2	19	40
Mean	21.3	38.9	169.2	69.3	24	36.2
SE +/-	0.4	0.98	1.5	3.4	0.97	7.6

BMI: Body Mass Index = body weight (kg) ÷ height^2 (m).

SOS: Sum of 5 Skinfolds (mm) = triceps + biceps + subscapular + iliac crest + medial calf (CSTF 1986).

Diet Analysis

A summary of the various nutrients comprising subjects' usual diet and the fat-reduced diet intervention is presented in Table 4.2. A breakdown of each macronutrient including energy intake is provided in Tables 4.3 through 4.15 and Figures 4.1 through 4.6.

Table 4.2 **Average daily composition of subjects' baseline vs fat-reduced diet (mean ± SE)**

Nutrient	Baseline Diet	Fat-reduced Die
Energy (kcal)	2251 ± 129.7	2234.1 ± 123.0
Protein (%)	14.4 ± 0.5	15.1 ± 0.2
Carbohydrate (%) §	54.3 ± 1.5	74.4 ± 0.2
Fat (%) §	30.8 ± 1.5	10.6 ± 0.2
Protein (g)	86.3 ± 4.8	84.5 ± 5.2
CHO (g) §	313.3 ± 21.8	430.3 ± 23.7
Fat (g) §	79.3 ± 7.9	27.4 ± 1.6
Saturated (g) §	31.5 ± 3.1	7.8 ± 0.6
Monounsaturated (g) §	28.3 ± 3.2	7.5 ± 0.4
Polyunsaturated (g) §	14.5 ± 1.5	4.9 ± 0.1
Cholesterol (mg) §	240.9 ± 33.2	48.2 ± 3.5
Fiber (g) §	19.4 ± 1.7	33.0 ± 1.8

comparison by student's t-test
§ $P < 0.05$

Energy intake of each individual during the fat-reduced diet was within 110 calories of their usual intake. Mean energy intake of subjects' usual diet was 2251 ± 130 kcal (range = 1667 - 2964 kcal) compared to 2234 ± 123 kcal (range = 1679 - 2875 kcal) for the fat-reduced diet. These were not significantly different.

Protein content of both diets represented approximately 15% of total calorie intake. Average % protein of subjects' usual and low-fat diet was 14.4 ± 0.45 and 15.1 ± 0.23 respectively (no significant difference).

Table 4.3 **Energy intake (kcal) of baseline (B) vs fat-reduced (FR) diet**

Subject	B	FR	Δ
1	2702	2766	64
2	1874	1954	80
3	2613	2510	-103
4	1918	1955	37
5	2064	1954	-110
6	2964	2875	-89
7	1667	1679	2
8	2391	2371	-20
9	2108	2064	-44
10	2209	2213	3
Mean	2,251.0	2,234.1	-18.0
SE ±	129.7	123.0	21.5

Table 4.4 **Caloric distribution of protein (%); baseline (B) vs fat-reduced (FR) diet**

Subject	B	FR	Δ
1	13	14	1
2	14	15	1
3	13	15	2
4	14	15	1
5	16	16	0
6	16	16	0
7	12	15	3
8	15	15	0
9	15	16	1
10	16	14	- 2
Mean	14.4	15.1	0.7
SE ±	0.4	0.2	0.4

Table 4.5 Daily protein intake (gm); baseline (B) vs fat-reduced (FR) diet

Subject	B	FR	Δ
1.0	85.4	90.5	5.1
2.0	70.0	75.7	5.7
3.0	87.6	95.0	7.4
4.0	72.5	76.3	3.8
5.0	96.3	80.1	-16.2
6.0	108.5	118.6	10.1
7.0	76.8	63.5	-13.3
8.0	95.6	96.4	0.8
9.0	64.1	66.0	1.9
10.0	106.1	83.2	-22.9
Mean	86.3	84.5	-1.8
SE ±	4.8	5.2	3.6

Table 4.6 Daily protein intake (gm/kg/day); baseline (B) vs fat-reduced (FR) diet

Subject	B	FR	Δ	% Δ
1	1.02	1.08	0.04	4
2	1.16	1.25	0.09	7
3	1.53	1.65	0.12	7
4	1.08	1.14	0.04	4
5	1.3	1.08	-0.22	-17
6	1.51	1.64	0.13	8
7	1.11	0.92	-0.19	-17
8	1.25	1.26	0.01	1
9	0.77	0.79	0.02	3
10	2.11	1.66	-0.45	-22
Mean	1.28	1.25	-0.04	-2.2
SE	0.12	0.1	0.06	3.7

Average grams of protein normally consumed was 86.3 ± 4.8 grams compared to 84.5 ± 5.2 grams during the fat-reduced diet intervention. Grams of protein consumed per kilogram of body weight per day was 1.28 ± 0.12 during the normal diet and 1.25 ± .01 during the fat-reduced diet.

Further examination of subjects' baseline dietary intake reveals the percentage of calories consumed as fat ranged from 26% - 39% (mean = 30 ± 1.47). The change in fat content constituted a 15 - 28% drop in percentage of calories consumed as fat (mean = 20 ± 1.38) for individuals, depending upon their usual dietary intake. In terms of grams consumed, subjects normally consumed, on average, between 51.7 and 116.6 grams of fat per day (mean = 79.3 ± 7.9 grams). During diet intervention, fat consumption ranged from 22.1-36.7 grams of fat per day (mean = 27.4 ± 1.6 grams), a significant drop of 51.9 ± 6.5 grams ($p < 0.05$). The significant decrease in fat content was accompanied by a significant increase in carbohydrate content ($p < 0.05$). Carbohydrate consumption increased from 313.3 ± 21.8 grams to 430.3 ± 23.7 grams. Percent of calories consumed as carbohydrates increased from an average of 54 ± 1.5 % to an average of 75 ± 0.2 %.

Table 4.7 Caloric distribution of fat (%); baseline (B) vs fat-reduced diet (FR)

Subject	B	FR	Δ
1	37	10	25
2	26	11	15
3	39	11	28
4	31	10	21
5	28	11	17
6	27	10	17
7	32	11	21
8	26	11	15
9	28	10	18
10	34	11	23
Mean	30.8	10.6	20.0 §
SE ±	1.5	0.2	1.4

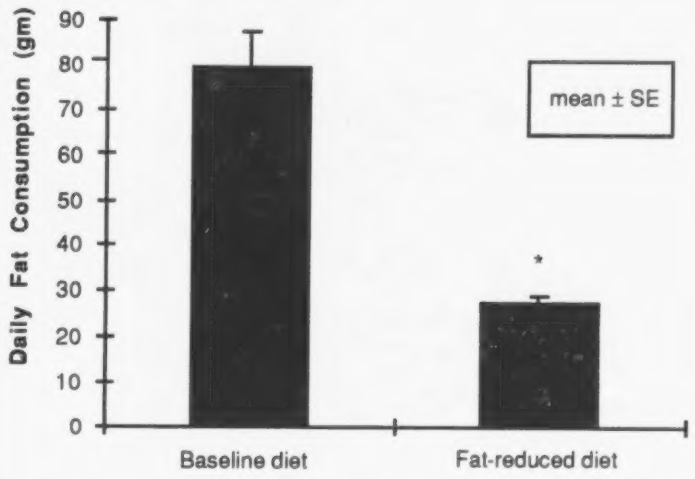
§ $P < 0.05$

Table 4.8 Daily fat intake (gm); baseline (B) vs fat-reduced (FR) diet

Subject	B	RD	Δ (gm)	% Δ
1	111.7	36.7	75.0	67
2	56.6	23.9	32.7	58
3	116.6	32.7	83.9	72
4	66.5	22.2	44.3	67
5	65.7	24.3	41.4	63
6	94.5	32.3	62.2	66
7	51.7	22.1	29.6	57
8	73.0	25.4	47.6	65
9	53.1	25.5	27.6	52
10	103.7	28.7	75.0	72
Mean	79.3	27.4	51.9 §	63.9
SE ±	7.9	1.6	6.5	2.1

§ $P < 0.05$

Figure 4.1 Daily Fat Consumption (gm)



(* $P < 0.05$)

Table 4.9 Caloric distribution of CHO (%); baseline (B) vs fat-reduced (FR) diet

Subject	B	FR	Δ
1	50	76	26
2	60	75	15
3	48	74	26
4	55	75	20
5	58	74	16
6	59	75	16
7	56	74	18
8	59	74	15
9	48	75	27
10	50	75	25
Mean	54.3	74.7	20.4 §
SE ±	1.5	0.2	1.6

§ = $P < 0.05$

Table 4.10 Daily CHO intake (gm); baseline (B) vs fat-reduced (FR) diet

Subject	B	FR	Δ (gm)	% Δ
1.0	341.0	524.2	183.2	35
2.0	279.6	379.6	100.0	26
3.0	315.5	481.6	166.1	35
4.0	275.3	384.0	108.7	28
5.0	305.0	374.0	69.0	18
6.0	466.3	560.6	94.3	17
7.0	201.5	322.8	121.3	38
8.0	350.5	456.4	105.9	23
9.0	272.0	387.0	115.0	30
10.0	326.7	433.0	106.3	25
Mean	313.3	430.3	117.0 §	27.5
SE ±	21.8	23.7	10.7	2.3

§ = $P < 0.05$

Baseline dietary fiber intake varied widely amongst subjects with a range of 9 - 27 grams of fiber per day (mean = 19.4 ± 1.7). This varied significantly with the fiber content of the fat-reduced diet which ranged from 25 - 43 grams of fiber per day (mean = 33 ± 1.8, $p < 0.05$) and represents approximately a 40% increase in fiber.

A breakdown of total fat consumed during the baseline diet and the fat-reduced diet into saturated, poly- and monounsaturated fats and cholesterol revealed the following:

1) Mean saturated fat consumption decreased from 31.5 ± 3.1 grams to 7.8 ± 0.6 grams; a significant change of 23.7 ± 2.6 grams ($p < 0.05$). This represented an approximate 74% change.

2) Mean monounsaturated fat consumption decreased from 28.3 ± 3.2 grams to 7.5 ± 0.4 grams; a significant change of 20.8 ± 2.9 grams ($p < 0.05$). This represented an approximate 72% change.

3.) Mean polyunsaturated fat consumption decreased from 14.5 ± 1.5 grams to 4.9 ± 0.1 grams; a significant change of 62.8 ± 3.7 gms ($p< 0.05$). This represented an approximate 63% change.

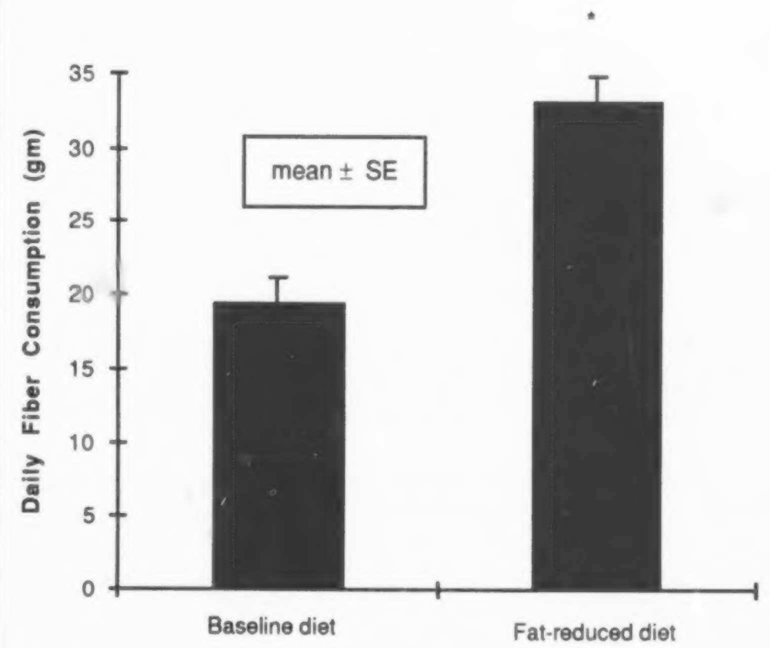
4.) Mean cholesterol consumption decreased from 240.9 ± 33.2 grams to 48.2 ± 3.5 grams; a significant change of 192.7 ± 31.1 ($p < 0.05$). This represented an approximate 78% change.

Table 4.11 **Daily fiber intake (gm); baseline (B) vs fat-reduced (FR) diet**

Subject	B	FR	Δ (gms)	% Δ
1	17	39	22	56
2	27	30	3	10
3	19	43	24	56
4	26	30	4	13
5	25	30	5	17
6	18	38	20	53
7	9	26	17	65
8	20	35	15	43
9	16	25	9	36
10	17	34	17	50
Mean	19.4	33.0	13.6 §	39.9
SE ±	1.7	1.8	2.5	6.3

§ = $P < 0.05$

Figure 4.2 Daily Fiber Consumption (gm)



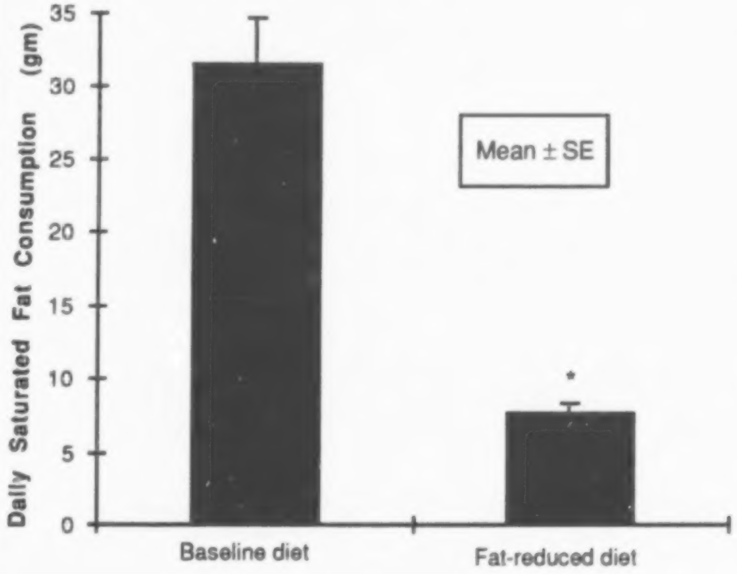
(*P< 0.05)

Table 4.12 Daily saturated fat intake (gm); baseline (B) vs fat-reduced (FR) diet

Subject	B	FR	Δ (gms)	% Δ
1	46.3	10.7	-35.6	77.0
2	19.8	6.5	-13.3	67.0
3	45.5	11.2	-34.3	75.0
4	36.3	5.4	-30.9	85.0
5	22.3	6.0	-16.3	73.0
6	32.1	8.1	-24.0	75.0
7	25.9	7.1	-18.8	73.0
8	28.5	8.8	-19.7	69.0
9	21.0	6.0	-15.0	71.0
10	37.6	8.1	-29.5	78.0
mean	31.5	7.8	§ -23.7	74.3
SE ±	3.1	0.6	2.6	1.6

§ $p < 0.05$

Figure 4.3 Daily Saturated Fat Consumption (gm)



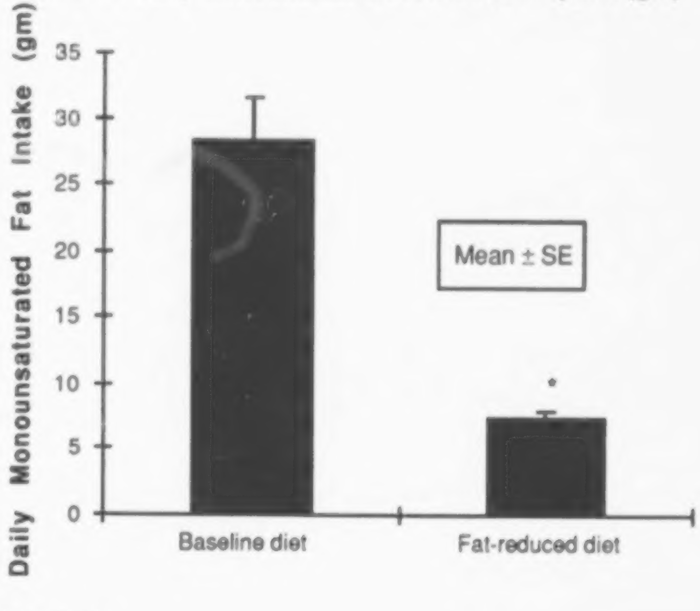
(P < 0.05)

Table 4.13 **Daily monounsaturated fat intake (gm); baseline (B) vs fat-reduced (FR) diet**

Subject	B	FR	Δ (gm)	% Δ
1	39.9	7.7	32.2	81
2	20.7	5.9	14.8	72
3	43.2	10.0	33.2	77
4	20.0	5.7	14.3	72
5	23.7	6.5	17.2	73
6	35.4	8.1	27.3	77
7	19.6	8.2	11.4	58
8	24.2	7.6	16.6	69
9	16.7	7.3	9.4	56
10	39.6	7.7	31.9	81
Mean	28.3	7.5	20.8 §	71.6
SE ±	3.2	0.4	2.9	2.7

§ = $P < 0.05$

Figure 4.4 Daily Monounsaturated Fat Consumption (gm)



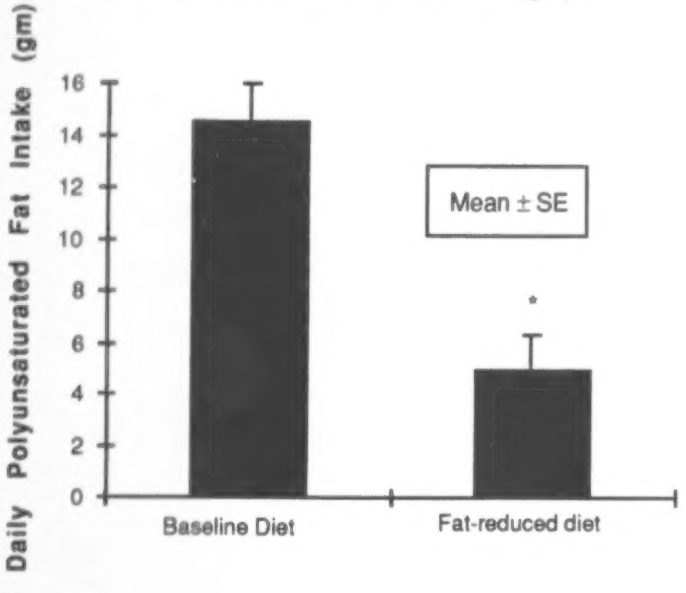
($P < 0.05$)

Table 4.14 Daily polyunsaturated fat intake (gm); baseline (B) vs fat-reduced (FR) diet

Subject	B	FR	Δ (gm)	% Δ
1	19.0	5.5	-13.5	71
2	9.6	4.2	-5.4	56
3	16.6	4.4	-12.2	73
4	16.1	5.1	-11.0	68
5	14.6	4.9	-9.7	66
6	21.5	5.7	-15.8	73
7	7.5	4.7	-2.8	37
8	12.4	4.9	-7.5	60
9	9.6	4.7	-4.9	51
10	18.2	5.0	-13.2	73
Mean	14.5	4.9	9.6 §	62.8
SE ±	1.5	0.1	1.4	3.7

§ $P < 0.05$

Figure 4.5 Daily Polyunsaturated Fat Intake (gm)



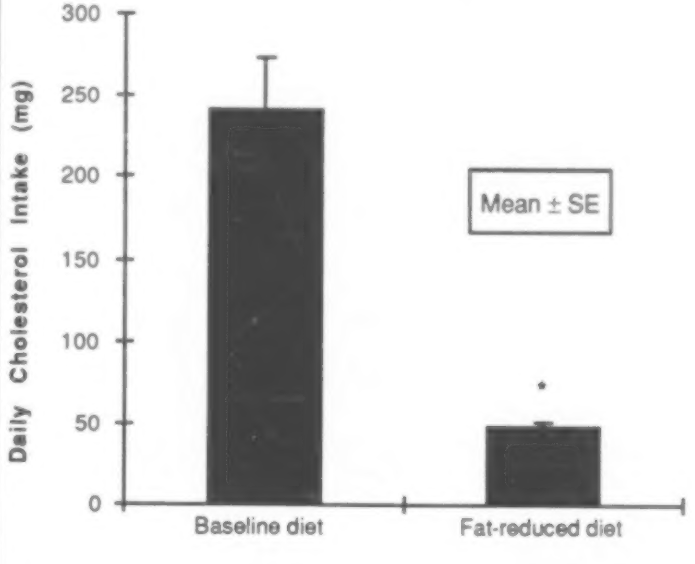
(*$P < 0.05$)

Table 4.15 Daily cholesterol intake (mg); baseline (B) vs fat-reduced (FR) diet

Subject	B	FR	Δ (mg)	% Δ
1	336.5	67.8	268.7	80
2	167.6	38.5	129.1	77
3	303.8	54.0	249.8	82
4	141.3	34.4	106.9	76
5	140.9	35.6	105.3	75
6	334.5	54.5	280.0	84
7	139.5	56.2	83.3	60
8	279.1	53.1	226.0	81
9	146.9	38.0	108.9	74
10	418.5	49.8	368.7	88
Mean	240.9	48.2	192.7 §	77.7
SE ±	33.2	3.5	31.1	2.4

§ = $P < 0.05$

Figure 4.6 Daily Cholesterol Intake (mg)



(*$P < 0.05$)

COMPARISON OF PRE- AND POST-DIET VARIABLES

Weight

Weight change following 5-day diet intervention was not significant and ranged from -2.4 to 1.5 kg (mean = -0.6 ± 0.4 kg) (Table 4.14).

Length of Menstrual Cycle

Menstrual Cycle 1 (during baseline dietary intake) ranged in length from 26-34 days (mean = 29.9 ± 0.8 days). Menstrual Cycle 2 (during which fat-reduced diet took place) ranged in length from 25-36 days (mean = 30.4± 1.0 days). Average change in menstrual cycle length was 0.4 ± 0.5 days and was not significant. Results are depicted in Table 4.15.

Table 4.16 **Change in weight (kg) immediately pre and post fat-reduced diet**

Subject	Pre-diet	Post-diet	Δ
1	83.4	84.2	0.8
2	59.8	59.8	0.0
3	57.3	57.0	-0.3
4	65.0	64.5	-0.5
5	71.4	69.0	-2.4
6	73.2	73.1	-0.1
7	69.3	67.4	-1.9
8	78.6	76.2	-2.4
9	82.3	83.8	1.5
10	52.3	51.5	-0.8
Mean	69.3	68.7	-0.6
SE ±	3.4	3.5	0.4

Table 4.17 **Menstrual cycle length (days); menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2)**

Subject	MC1	MC2	Δ
1	31	31	0
2	28	31	3
3	28	26	- 2
4	31	32	1
5	26	25	0
6	31	32	- 1
7	29	29	0
8	33	36	3
9	34	33	- 1
10	28	29	1
Mean	29.9	30.4	0.4
SE ±	0.8	1.0	0.5

Hormonal Analysis

Cortisol

Mean cortisol concentrations were not significantly different between menstrual cycle 1 (mean = 250 ± 27.9 nmol/L) and menstrual cycle 2 (mean = 240 ± 16.1 nmol/L). Mean cortisol decreased in 7 subjects and increased in 3 (Table 4.18 & Figure 4.7). Area under the curve was not significantly different between menstrual cycle 1 (mean = 2030 ± 226.2 nmol/L•hr) and menstrual cycle 2 (mean = 1910 ± 126.9 nmol/L•hr). Area under the curve decreased in 7 subjects and increased in 3 (Table 4.19 & Figure 4.7).

Table 4.18 Mean cortisol concentration (nmol/L); MFP of menstrual cycle1 (MC1) vs MFP of menstrual cycle2 (MC2). Hourly samples obtained over an 8 hour period.

Subject	MC1	MC2	Δ
1	250	240	-10
2	160	180	20
3	180	230	50
4	300	270	-30
5	250	220	-30
6	130	300	70
7	430	330	-100
8	310	250	-60
9	310	170	-140
10	220	200	-20
Mean	250	240	-25
SE ±	27.9	16.1	20.3

Figure 4.7 Mean cortisol concentration (μg/dL); menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). Hourly samples obtained over an 8 hour period. To convert μg/dL to nmol/L, multiply by 27.59.

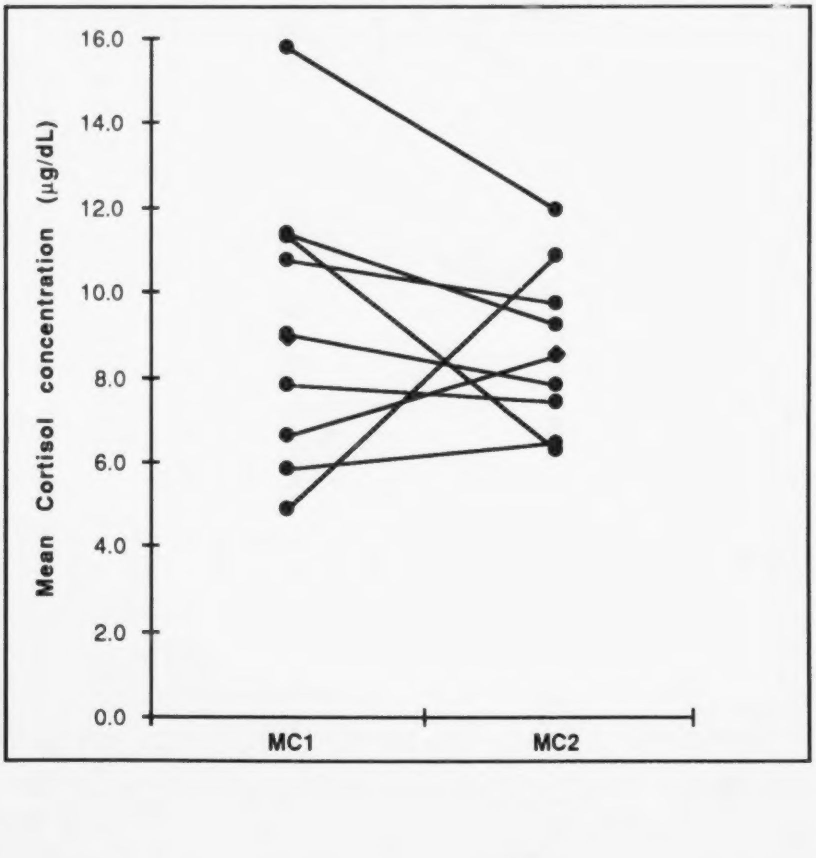


Figure 4.8 Mean cortisol concentrations (μg/dL); menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). Hourly samples obtained over an 8 hour period. To convert μg/dl to nmol/L, multiply by 27.59.

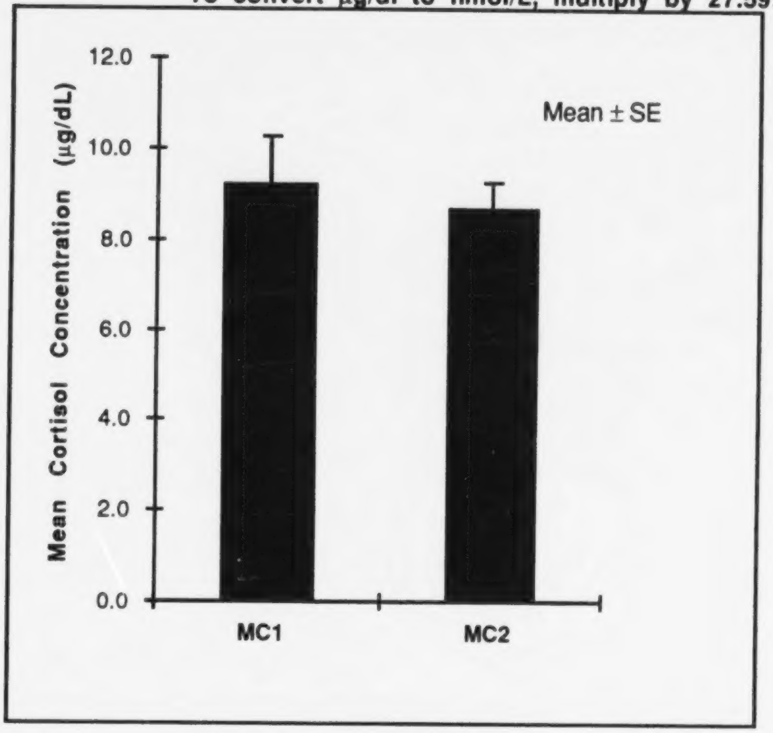


Table 4.19 Cortisol area under the curve (nmol/L•hr); MFP of menstrual cycle1 (MC1) vs MFP of menstrual cycle 2 (MC2). Hourly samples over an 8 hour period.

Subject	MC1	MC2	Δ
1	1970	1880	-90
2	1290	1420	130
3	1450	1880	430
4	2370	2150	-220
5	1980	1730	-250
6	1070	2380	1310
7	3480	2640	-840
8	2500	2030	-470
9	2490	1390	-1100
10	1720	1630	-90
Mean	2030	1910	-120
SE ±	226.2	126.9	213.2

Figure 4.9 Cortisol area-under-the-curve (μg/dL•hr); MFP of menstrual cycle 1 (MC1) vs MFP of menstrual cycle 2 (MC2). Hourly samples obtained over an 8 hour period. To convert μg/dL to nmol/L, multiply by 27.59.

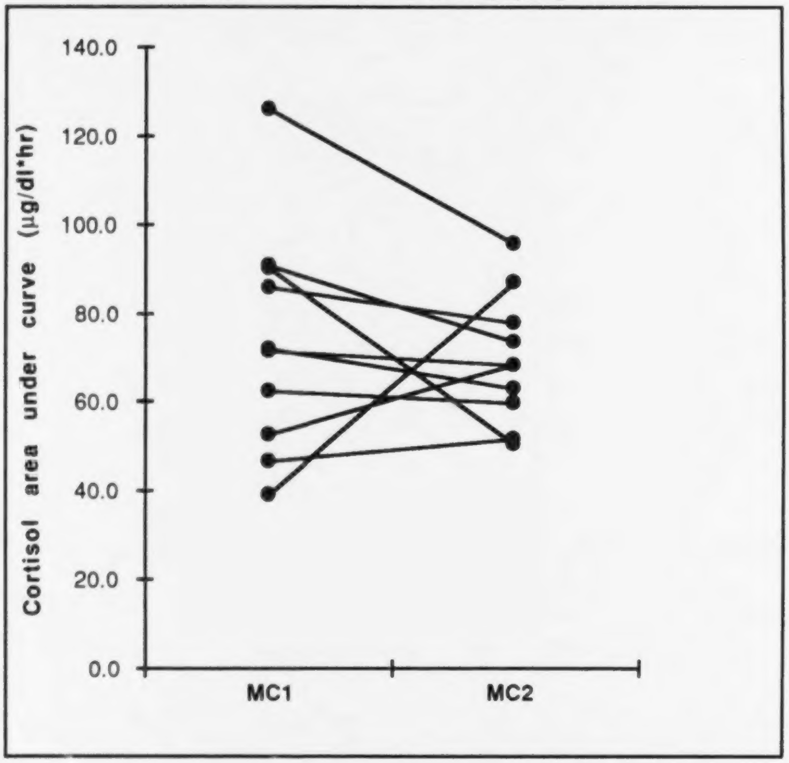
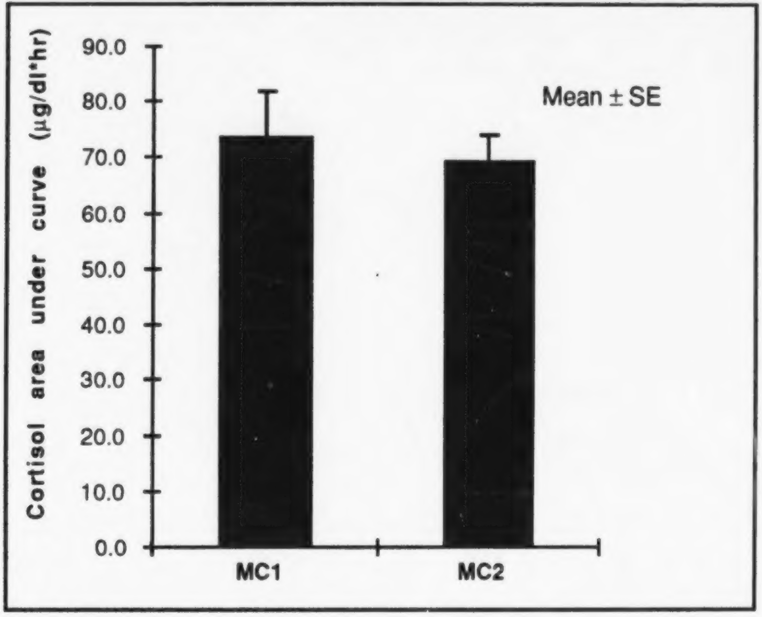


Figure 4.10 **Cortisol area-under-the-curve; menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). Hourly samples obtained over an 8 hour period. To convert μg/dL to nmol/L, multiply by 27.59.**



Luteinizing Hormone

LH mean concentration decreased in 6 subjects and increased in 4 subjects. There was no significant difference in mean LH concentrations between menstrual cycle 1 (mean = 2.05 ± 0.4 mIU/ml) and menstrual cycle 2 (mean = 1.92 ± 0.4 mIU/ml) (Table 4.20, Figures 4.11 & 4.12). Pulse frequency was not significantly different between menstrual cycle 1 (mean = 5.6 ± 0.2 pulses/8hrs) and menstrual cycle 2 (mean = 5.6 ± 0.3 pulses/8hrs) (Table 4.21, Figures 4.13 & 4.14). LH pulse amplitude was not significantly different between menstrual cycle 1 (mean = 1.0 ± 0.8 mIU/ml) and menstrual cycle 2 (mean = 1.1 ± 0.2 mIU/ml) (Table 4.22, Figures 4.15 & 4.16). There was no significant difference in LH area-under-the-curve between menstrual cycle 1 (mean = 55.9 ± 10.7 mIU/ml) and menstrual cycle 2 (mean = 55.5 ± 12.7 mIU/ml) (Table 4.23, Figures 4.17 & 4.18).

Table 4.20 Mean LH concentration mIU/ml; MFP of menstrual cycle1 (MC1) vs MFP of menstrual cycle 2 (MC2). 10 min samples obtained over an 8 hour period.

Subject	MC1	MC2	Δ
1	1.5	1.3	-0.2
2	3.9	2.6	-1.3
3	2.3	1.0	-1.2
4	1.4	1.1	-0.3
5	0.7	0.7	-0.1
6	3.1	3.7	0.6
7	3.7	4.7	1.0
8	2.0	1.6	-0.4
9	0.8	1.2	0.4
10	1.1	1.4	0.3
Mean	2.1	1.9	-0.1
SE ±	0.4	0.4	0.2

Figure 4.11 Mean LH concentration (mIU/ml); MFP of menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

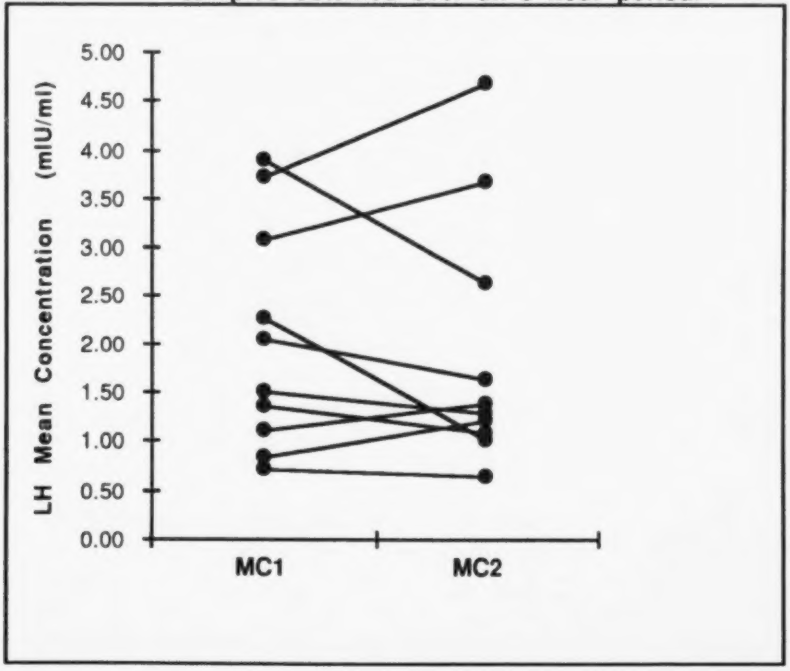


Figure 4.12 Mean LH concentration (mIU/ml); menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

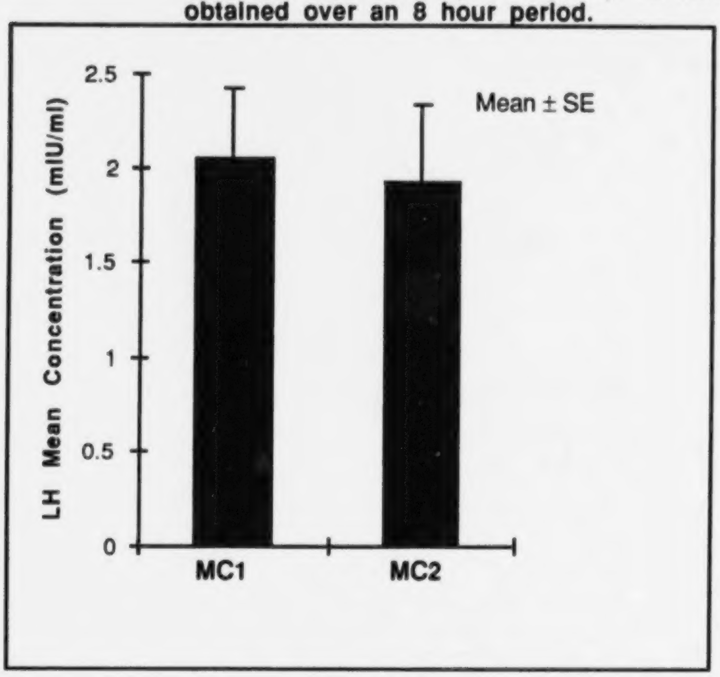


Table 4.21 LH frequency (#pulses/8 hr); MFP of menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

Subject	MC1	MC2	Δ
1	5.0	5.0	0.0
2	5.5	5.8	0.3
3	5.8	6.5	0.8
4	7.3	6.3	-1.0
5	5.5	5.0	-0.5
6	5.3	5.5	0.3
7	5.3	4.8	-0.5
8	6.3	7.0	0.8
9	4.8	5.8	1.0
10	5.5	4.3	-1.3
Mean	5.6	5.6	0.0
SE ±	0.2	0.3	0.2

Figure 4.13 LH frequency (#pulses/8 hr); MFP of menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

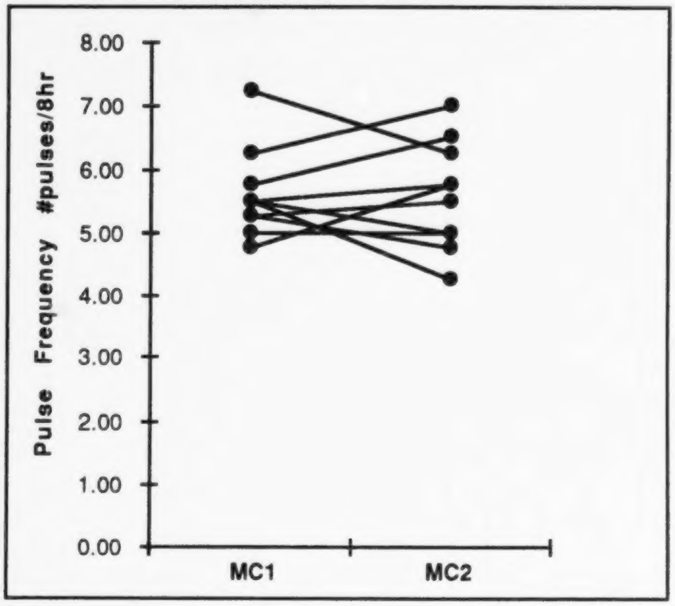


Figure 4.14 LH pulse frequency (#pulses/8 hr); MFP of menstrual cycle 1 (MC1) vs MFP of menstrual cycle 2. 10 minute samples obtained over an 8 hour period.

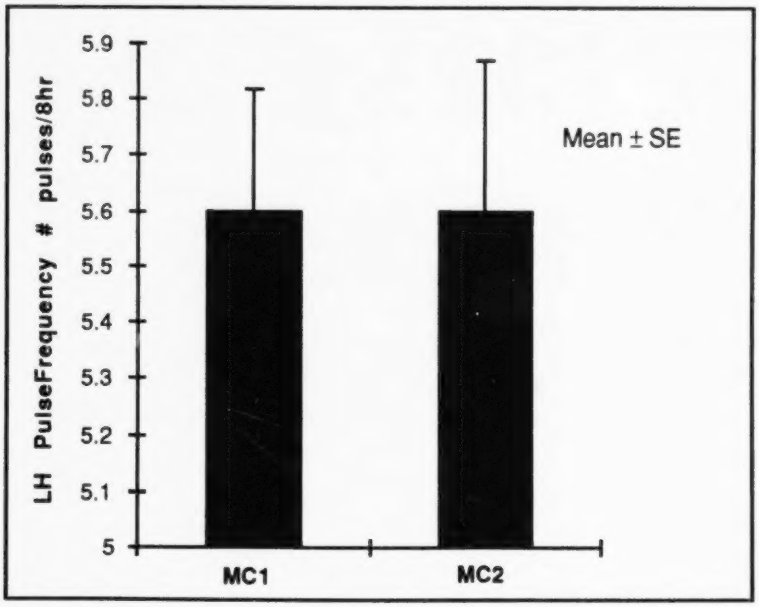


Table 4.22 LH pulse amplitude (mIU/ml); MFP of menstrual cycle1 (MC1) vs MFP of menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

Subject	MC1	MC2	Δ
1	0.6	0.4	-0.2
2	1.9	1.5	-0.4
3	1.3	0.4	-1.0
4	0.7	1.0	0.3
5	0.3	0.4	0.3
6	1.1	1.7	0.6
7	2.0	2.9	0.9
8	0.9	0.7	-0.2
9	0.9	0.9	0.0
10	0.7	1.1	0.4
Mean	1.0	1.1	0.1
SE ±	0.2	0.2	0.2

Figure 4.15 LH pulse amplitude (mIU/ml); MFP of menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

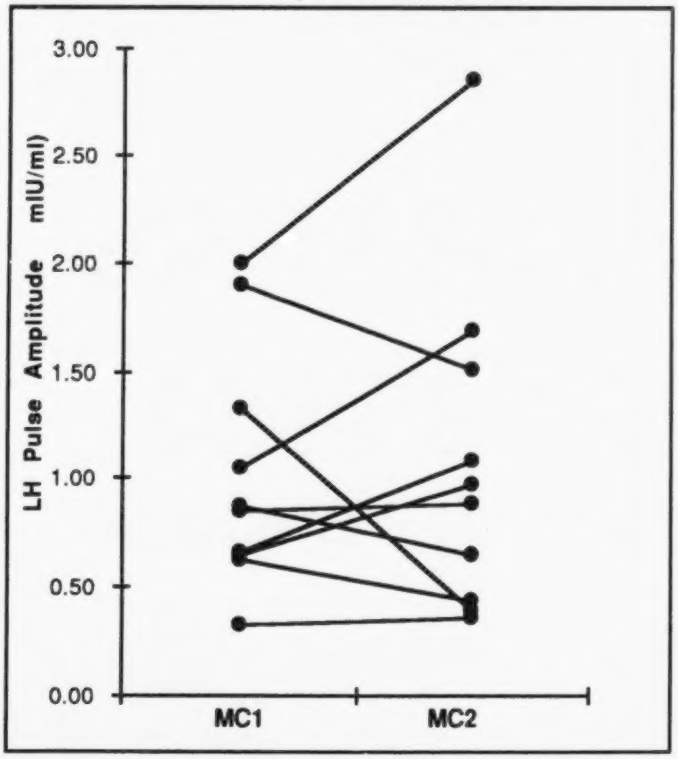


Figure 4.16 LH pulse amplitude (mIU/ml); MFP of menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). 10 minute samples over an 8 hour period.

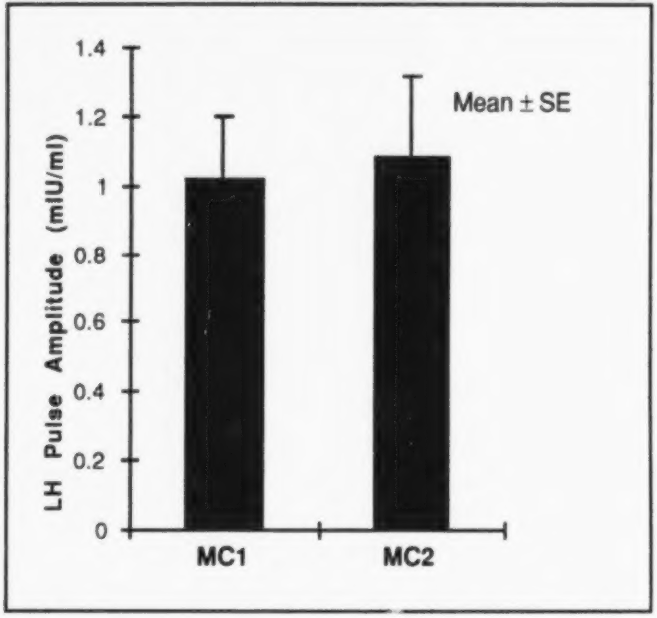


Table 4.23 LH area under the curve (mIU/ml•min); MFP of menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

Subject	MC1	MC2	Δ
1	31.2	20.3	-10.9
2	100.2	65.6	-34.6
3	72.6	19.1	-53.5
4	25.2	56.2	31.0
5	19.6	10.2	-9.4
6	53.6	74.4	20.8
7	123.6	141.5	17.9
8	34.6	27.3	7.3
9	50.0	50.3	0.2
10	48.2	89.7	41.5
Mean	55.9	55.5	1.0
SE ±	10.7	12.7	9.3

Figure 4.17 LH area-under-the-curve (mIU/ml·min); MFP of menstrual cycle1 (MC1) vs MFP of menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.

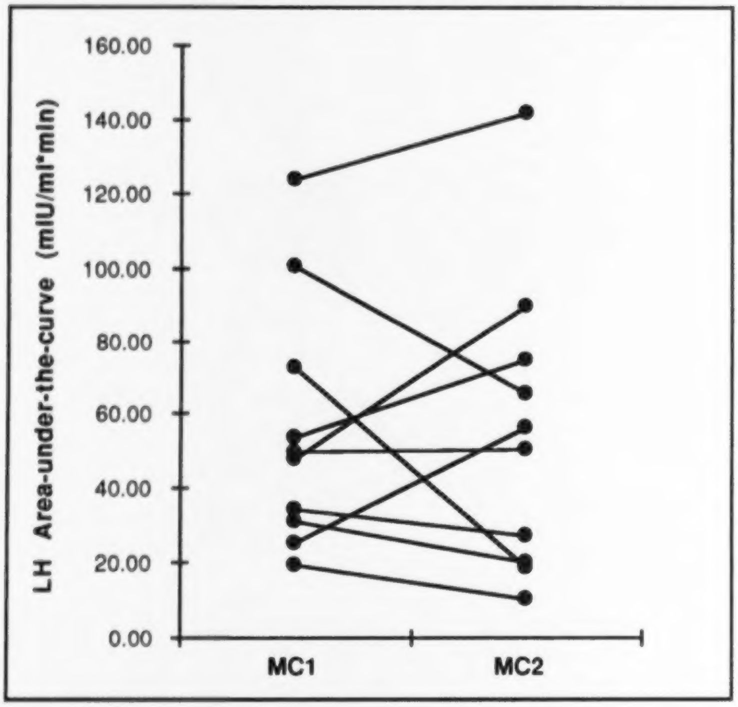
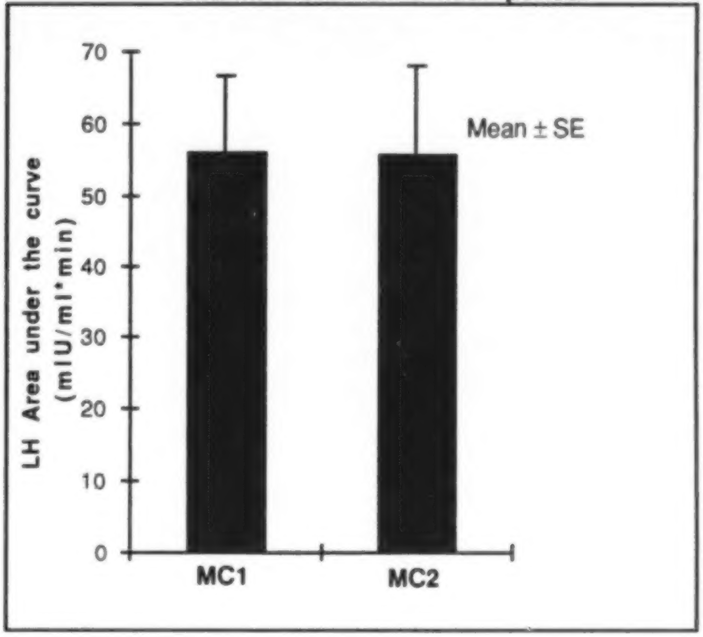


Figure 4.18 LH area-under-the-curve (mIU/ml•curve); MFP of menstrual cycle1 (MC1) vs MFP of menstrual cycle 2 (MC2). 10 minute samples obtained over an 8 hour period.



Progesterone

Incomplete progesterone data sets for 3 subjects necessitated the exclusion of these subjects from further data analysis. Out of 7 subjects, 6 experienced a drop in mean progesterone concentrations and progesterone area-under-the- curve. Both a *t* test and a non-parametric inference test (Wilcoxon matched-pairs ranks test) were used to analyze the progesterone data. Mean progesterone concentrations (n=7) were not significantly different between menstrual cycle 1 (mean = 711 ± 49.3 pmol/L) and menstrual cycle 2 (mean = 599 ± 79.2 pmol/L) (Table 4.24, Figures 4.19 & 4.20). No significant difference was detected in progesterone area-under-the-curve between menstrual cycle 1 (mean = 11,448 ± 916.5 pmol/L•day) and menstrual cycle 2 (mean = 9502 ± 980.7 pmol/L•day) (Table 4.25, Figures 4.21 & 4.22).

Table 4.24 Salivary progesterone mean concentration (pmol/L); menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). Daily AM samples, day 12 until onset of menses.

Subject	MC1	MC2	Δ
2	511	464	-47
4	664	624	-40
5	792	461	-331
7	602	543	-59
8	700	548	-152
9	860	500	-360
10	849	1057	208
Mean	711	599	-112
SE ±	49.3	79.2	74.7

Figure 4.19 Salivary progesterone mean concentration (pg/ml); menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2). Daily AM samples, day 12 until onset of menses. To convert pg/ml to pmol/L, multiply by 3.18.

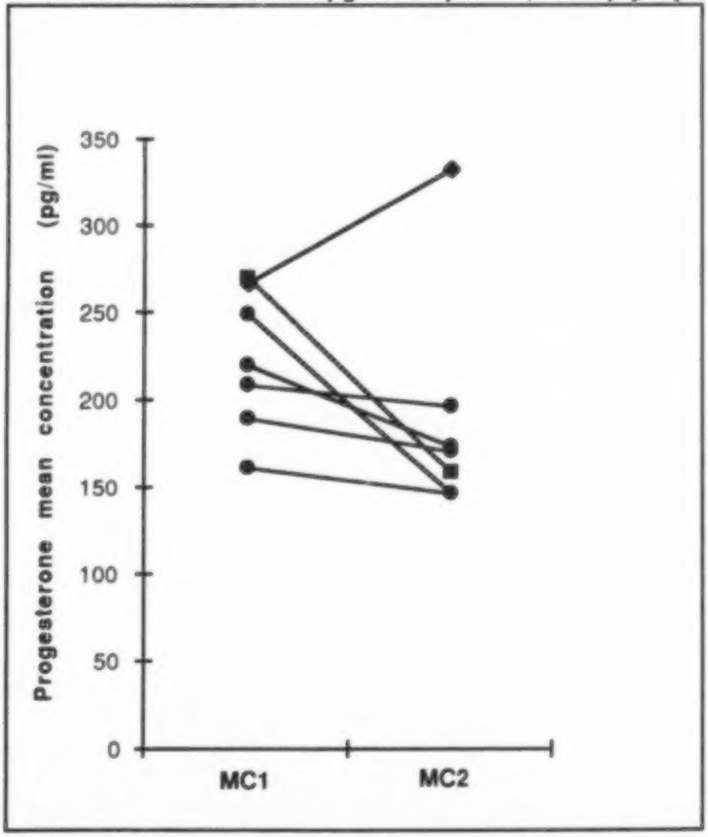


Figure 4.20 Salivary progesterone mean concentration; menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). Daily AM samples, day 12 until onset of menses. To convert pg/ml to pmol/L, multiply by 3.18.

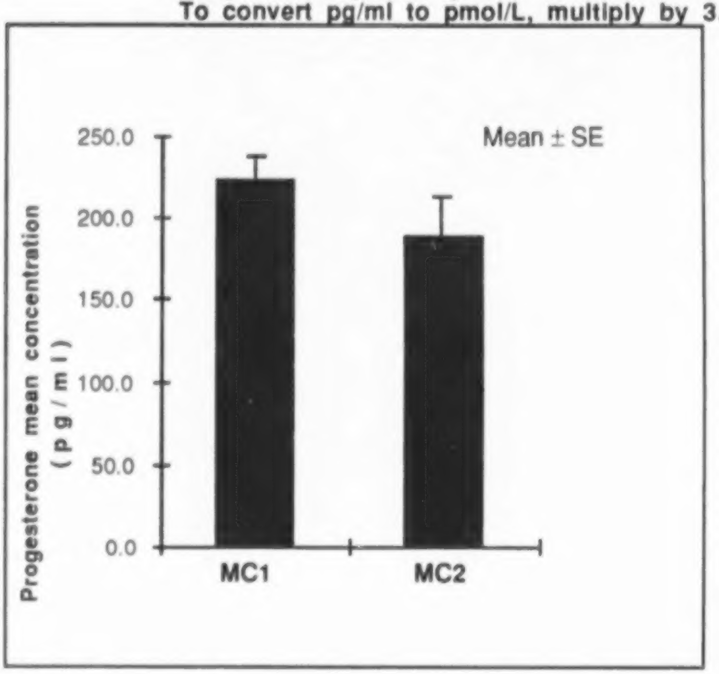


Table 4.25 Salivary progesterone area-under-the-curve (pmol/L•day); menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2). Daily AM samples, day 12 until onset of menses.

Subject	MC1	MC2	Δ
2	7672.0	6954.0	-718.0
4	12608.0	11851.0	-757.0
5	11877.0	6913.0	-4964.0
7	9027.0	8145.0	-882.0
8	13304.0	10407.0	-2897.0
9	14611.0	8498.0	-6113.0
10	11036.0	13747.0	2711.0
Mean	11448.0	9502.2	-1945.5
SE ±	916.5	980.7	1124.8

Figure 4.21 Salivary progesterone area-under-the-curve (pg/ml•day); menstrual cycle1 (MC1) vs menstrual cycle 2 (MC2). Daily AM samples, day 12 until onset of menses. To convert pg/ml to pmol/L, multiply by 3.18.

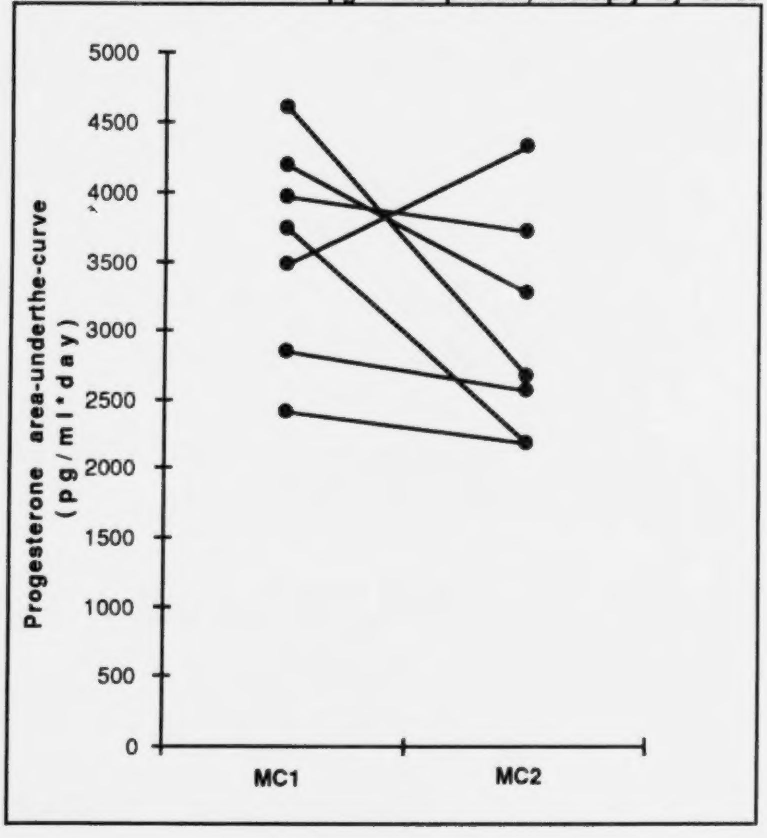


Figure 4.22 Salivary progesterone area-under-the-curve; menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2). Daily AM samples, day 12 until onset of menses. To convert pg/ml to pmol/L, multiply by 3.18.

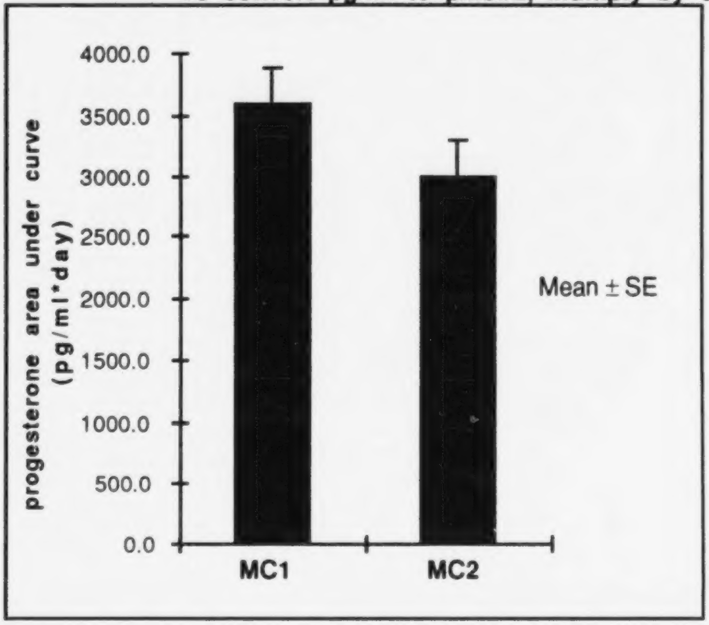


Table 4.26 Summary of hormonal responses: menstrual cycle 1 (MC1) vs menstrual cycle 2 (MC2).

Hormone	MC1	MC2
Mean Cortisol nmol/L	250 ± 27.6	240 ± 16.1
Cortisol Area/Curve nmol/L•day	2030 ± 226.2	1910 ± 126.9
Mean Salivary Progesterone pmol/L	711 ± 49.3	599 ± 79.2
Salivary Progesterone Area/Curve pmol/L•day	11448 ± 916.5	9502.2 ± 980.7
Mean LH mIU/ml	2.1 ± 0.3	1.9 ± 0.4
LH area/curve mIU/ml•min	55.8 ± 10.6	55.5 ± 12.7
LH Pulse Frequency #pulses/8hr	5.6 ± 0.2	5.6 ± 0.3
LH Pulse Amplitude mIU/ml	1.0 ± 0.2	1.1 ± 0.2

Pearson Product Moment Correlation

Pearson product moment correlation was calculated between changes in diet, hormone and body composition variables. The results are reported in Appendix 10.

The following were significantly correlated at the 0.05 level of significance (N = 10):

1. Carbohydrate intake was negatively correlated with saturated fat intake (r = -0.70) and positively correlated with fiber intake (r = 0.66).

2. Total fat intake was positively correlated with saturated fat intake (r = 0.85), monosaturated fat intake (r = 0.98), polyunsaturated fat intake (r = 0.83), cholesterol (r = 0.86) and negatively correlated with fiber intake (r = -0.74).

3. Saturated fat was positively correlated with mono- and polyunsaturated fats (r = 0.78 and 0.73).

4. Monounsaturated fats were positively correlated with polyunsaturated fats (r = 0.83).

5. Polyunsaturated fats were negatively correlated with mean cortisol and cortisol area-under-the-curve (r = -0.76).

6. Monounsaturated fats were negatively correlated with mean cortisol and cortisol area-under-the-curve (r = -0.64).

7. Protein was positively correlated with LH pulse frequency (r = 0.65).

8. Mean cortisol and cortisol area-under-the-curve were positively correlated with each other (r = .99).

9. Mean LH and LH area-under-the-curve were positively correlated with each other (r = 0.75).

10. LH pulse frequency and LH area-under-the-curve were negatively correlated (r = -0.61).

11. LH pulse amplitude was positively correlated with LH mean concentration ($r = 0.89$) and LH area-under-the-curve ($r = 0.83$).

Due to the reduced number of subjects ($N = 7$) used in the progesterone analysis, Pearson Product moment correlations between progesterone and other variables were calculated separately and reported in Appendix 10. Both mean progesterone and progesterone area-under-the-curve were significantly negatively correlated ($P < 0.05$) with BMI ($r = -0.85$).

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Canadian Association of Sport Sciences. 1986. Canadian Standardized Test of Fitness (CSTF) Operations Manual (3rd ed). p 8. Ottawa: Government of Canada.

CHAPTER FIVE

DISCUSSION

It has been well established that reproductive processes are influenced by nutritional status. Examples of reproductive failure in face of an energy deficit abound in both animal (Foster & Olster 1985, Bronson & Heideman 1990) and human studies (Van der Walt et al. 1978, Ehrenkranz et al. 1983). Breeding patterns of wild animals tend to be related to periods of food availability (Sadlier 1969, Bronson 1988a). In an evolutionary sense, these changes may represent reproductive strategies employed by the organism to conserve energy when food conditions are poor (Bronson 1988a). Only when the energy demands of foraging are well met, can the animal expend the extra energy required for growth and reproductive processes (L'anson et al. 1991).

While it is evident that caloric intake represents an important variable in reproductive success, the role of specific macronutrients (carbohydrates, protein and fat) has yet to be determined. Research has recently focused upon vegetarian populations which demonstrate a high incidence of menstrual dysfunction and infertility (Hill 1984 et al., Lloyd 1991 et al., Pedersen et al. 1991). Hormonal patterns displayed by vegetarian women differ from those consuming a mixed diet (Goldin et al. 1982, Pirke et al. 1986). Vegetarian diets tend to be lower in total fat, saturated fat and cholesterol and higher in fiber (Pedersen, 1990). As well, restriction of dietary fat has become a method of weight reduction employed by many women (Loucks 1988). Popular weight reduction programs are now extolling the benefits of extremely low fat, high fiber dietary plans, advising participants to limit fat consumption to < 35 grams of fat per day regardless of calorie intake. Assuming an individual was consuming 2000 cal/day, 35 grams of fat would constitutute < 16% of total calorie intake;

far below the recommended daily allowance of 30% suggested by Health and Welfare Canada (1990). Owing to the popularity of such diet fads and the possibility of reproductive repercussions, the role of dietary fat in maintaining normal menstrual periodicity needs to be established. The purpose of this project was to determine if acute reductions in dietary fat are associated with changes in LH pulsatility and progesterone, (considered to be reliable markers of reproductive function), as well as changes in cortisol profiles in young women.

Luteinizing Hormone

Administration of an acute low-fat diet to young eumenorrheic women appears to have had no significant effect upon mid-follicular phase LH secretion. LH pulse frequency, pulse amplitude, mean concentration and area-under-the-curve showed no consistent change in response to changes in dietary fat content. Mean luteinizing hormone concentrations both pre-and post-diet were all within the assay predicted range for females in the follicular phase of the menstrual cycle (0.6-6.2 mIU/ml). If normal episodic release of luteinizing hormone during the mid-follicular phase of the menstrual cycle is approximately 4 pulse/6 hrs or .66 pulses/hr (Backstrom 1982), then 5.3 pulses can be expected within an 8 hour period. In this study, a mean frequency of approximately 5.6 pulses was recorded during both the control menstrual cycle and the diet cycle.

The effect of energy restriction upon LH secretion has been well documented. Frequency and amplitude of LH pulses are dramatically reduced in the starving animal yet rapidly resume upon refeeding (Foster & Olster 1985, McCann & Hansel 1986, Bronson & Heideman 1990). Immature LH pulse patterns are displayed by anorexic individuals (Boyar et al. 1974).

The site at which these changes occur is likely the hypothalamus. Administration of exogenous GnRH to starving rats has been shown to initiate puberty despite a total lack of growth (Bronson1986, 1988b). As well, normal plasma levels of LH can be reestablished in starving rhesus monkeys via a GnRH replacement regimen (Dubey et al. 1986). Similarly, administration of exogenous GnRH to anorexic women results in resumption of normal LH secretory patterns (Wiegelmann & Solbach 1972). It seems likely that GnRH release and not GnRH synthesis is affected by poor nourishment. Post-mortem measurements of GnRH content within the median eminence of starving rats are no different from rats which are well-fed (Bronson 1988b).

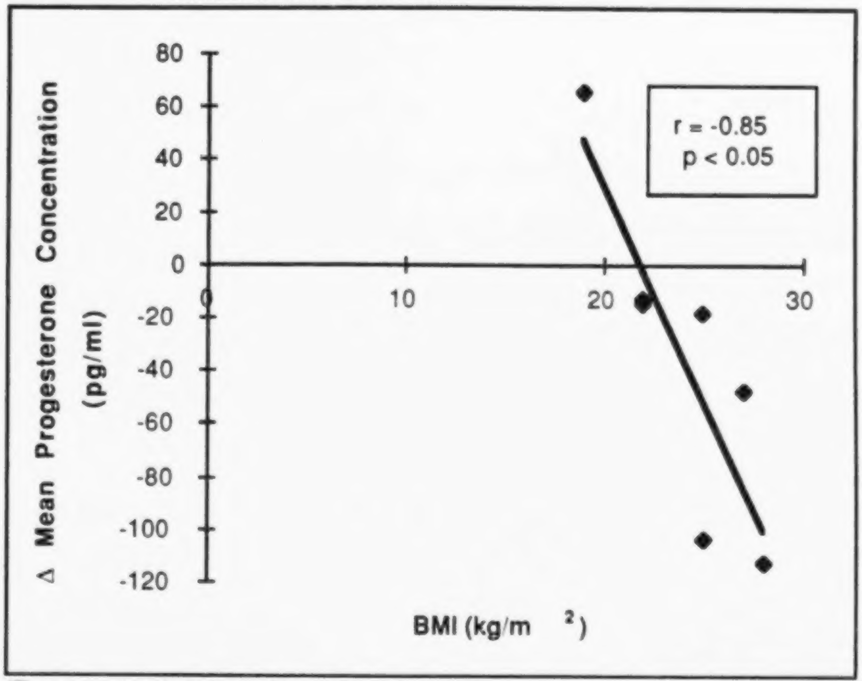
The negative results in this investigation concur with the findings of Cameron (Cameron 1989), who was unable to detect differences in LH concentrations in rhesus monkeys subjected to an 8-week, low-fat diet regimen. Similarly, Hagerty et al. (1988) detected no differences in LH concentrations in young women placed on a 25% fat diet for a 1 month period. Hill et al. (1984) studied the effects of vegetarianism on gonadotropin release. Contrary to the previous findings, these researchers demonstrated a decrease in episodic LH release in omnivorous women when placed on a vegetarian diet in which fat content was decreased from 38% to 33% of total calorie intake. The longer length of the diet imposed by the Hill research (2 months as opposed to 5 days) may have been instrumental in eliciting changes in LH not demonstrated in the present study, despite a much smaller decrease in fat content. It is also possible that manipulation of some other dietary constituent elicited the changes seen in the Hill study. Hill et al. (1986) demonstrated that the addition of meat to the diets of vegetarian women increases LH frequency, LH mean concentration and area-under-the-curve. A soybean supplement of equal protein content failed to elicit these changes. It is possible that the removal or addition of animal protein was responsible for the changes in LH pulsatility. The relationship between type of protein and reproductive function has yet to be researched extensively. However, limiting the consumption of a single essential amino acid (valine) has been shown to delay puberty and reduce gonadotropin levels in rats (Glass & Swerdloff 1977).

Progesterone

Subjects were entrusted to collect salivary samples daily and store samples in their home freezer. Unfortunately, several samples were missing from the sets collected by 3 subjects. Therefore, it was felt necessary to eliminate these 3 subjects from the progesterone analysis.

Salivary progesterone concentrations agreed with values (100-200 pg/ml or 300-600 pmol/L) expected during the luteal phase of the menstrual cycle (Choe et al. 1983, Zorn et al. 1984, Wang et al. 1985, Vuorento et al. 1989). Although, no significant differences were detected for mean progesterone concentration or area-under-the-curve following low-fat diet intervention, 6 out of 7 subjects did experience a drop in both of these parameters. Changes in mean concentration and area-under-the-curve were negatively correlated ($r = -0.85$, $p < 0.05$, $N = 7$) with BMI (Figure 5.1). Subjects with a higher BMI tended to display greater decreases in progesterone concentration and area-under-the-curve when subjected to a reduced-fat diet. The relevance of this association is unknown, particularly since a relationship could not be established between progesterone concentrations and sum-of-skinfolds. A relationship did not exist between BMI and normal diet progesterone levels ($r = 0.13$). However, the correlation between BMI and reduced-fat diet salivary progesterone concentrations ($r = -0.71$) approaches significance. This is interesting in that it suggests that the heavier women in the study responded differently, than their lighter counterparts, to some aspect of the diet intervention.

Figure 5.1 Correlation between Δ in mean salivary progesterone concentration and BMI



(To convert pg/ml to pmol/L, multiply by 3.18).

Reproductive disorders have previously been related to low body weight and limited fat reserves (Frisch & McArthur 1974). Suppression of ovarian function has been noted in relation to weight loss (Panter-Brick et al. 1993). Thus, it is somewhat surprising that in this study, heavier women showed the greatest decreases in progesterone concentrations when subjected to an extreme dietary fat reduction. Differences cannot be attributed to weight change, as this was minimal for all subjects over the 5 day period. It is well established that obese women display a high prevalence of menstrual disorders (Edman & MacDonald 1978, O' Dea et al. 1979). Normalization of hormonal parameters has been shown to occur following weight loss (O'Dea et al. 1979, Kiddy et al. 1992, Gusick et al. 1994). It is reasonable to suspect that heavier individuals respond differently, in a metabolic sense, to changes in diet. It may be worthwhile investigating this relationship further, using a more tightly matched subject group in terms of body composition variables.

The effects of dietary change on progesterone concentrations has been investigated by a number of researchers. Hagerty et al. (1988) reported no difference in serum progesterone, LH or estradiol levels in response to either a 1 month, low-fat diet intervention (25% fat) or a 1 month, high-fat diet (40% fat) intervention. However, Pirke et al. (1986) report decreased luteal phase progesterone concentrations and increased incidence of anovulatory cycles in response to a low-calorie vegetarian diet; changes which were not noted in women consuming a low-calorie mixed diet. The vegetarian group in the PIrke study experienced a greater change in fat content than the nonvegetarian group (14% change as opposed to 8%). However, calorie count, protein content and carbohydrate content of the two groups' diets also varied considerably, confounding efforts to identify the precise dietary aspect responsible for the observed results.

Decreased progesterone concentrations are usually indicative of corpus luteum defect due to inadequate luteinizing of granulosa cells by LH during the follicular phase of the menstrual cycle (Sherman & Korenman 1974). The !Kung Bushwomen of Botswana display both lowered progesterone and estrogen levels in comparison to urbanized South African negro women (Van der Walt et al. 1978). This hunting / foraging Kalahari Desert tribe experiences seasonal birth fluctuations in accordance with food availability. Deficiency in calories and a number of other undetermined nutrients is likely during the dry season. When tested, progesterone levels indicative of a functioning corpus luteum were present in only 1 out of the 19 !Kung women tested.

There was no attempt to assess corpus luteal function in this particular study. However, the fact that salivary progesterone concentrations decreased in 6 out of 7 women deserves further consideration. Unfortunately, a significant correlation between progesterone concentrations and any of the dietary variables could not be established.

Cortisol

Mean concentrations of cortisol and area-under-the-curve for an 8 hour period were not significantly different following the 5-day, fat-reduced diet. Interestingly, 7 out of 10 subjects experienced a decrease in cortisol concentrations. Elevated cortisol concentrations in combination with restricted food intake have been well documented (Boyar et al. 1977, Caspar et al. 1979, Gold et al. 1986). However, little information is available on the effects of fat restriction on the adrenal axis.

For this study, all values in the present study were within expected limits (A.M. values: 3-20 µg/dl (80 - 550 nmol/L), P.M. values: half of A.M. values) (Aron & Tyrell, 1994). A negative correlation ($r = -0.58$) between change in grams of fat and change in mean cortisol concentrations and area-under-the-curve approached significance. Both changes in mono- and polyunsaturated fats were significantly, negatively correlated with changes in cortisol measurements ($r = -0.64$ and -0.76 respectively, $p < 0.05$, $N = 10$). Figures 5.2 and 5.3 illustrate the inverse relationship between these variables. Owing to variability in normal fat intake, absolute change in fat intake differed between individuals. In general, subjects who experienced the greatest decreases in unsaturated fats, tended to demonstrate greater increases in cortisol concentrations.

Figure 5.2 Correlation between Δ in cortisol area-under-the curve and Δ in polyunsaturated fat intake.

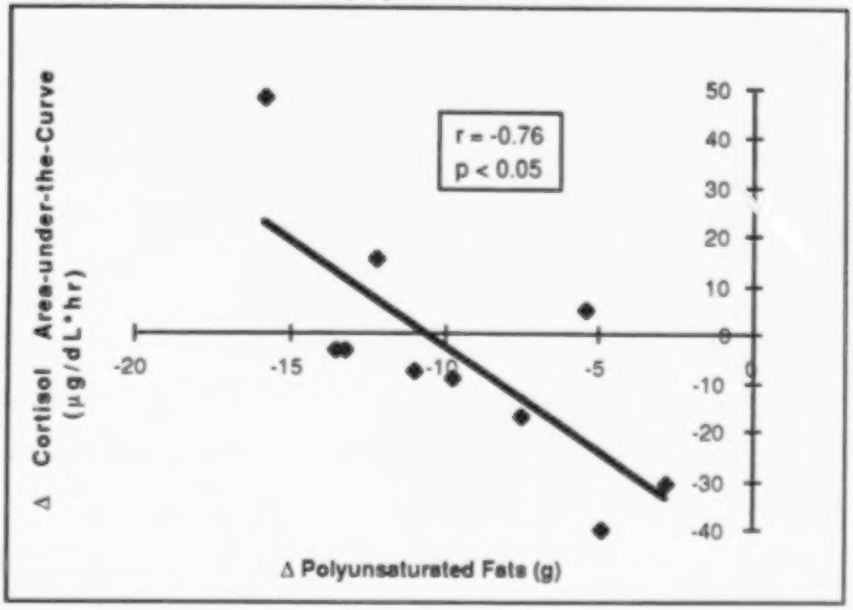
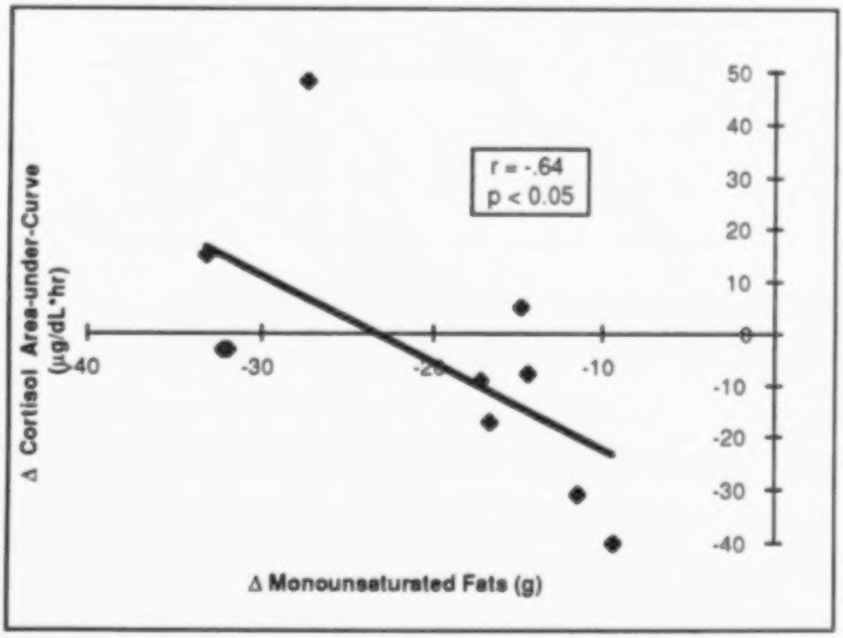


Figure 5.3 Correlation between Δ in cortisol area-under-the-curve and Δ in monounsaturated fat intake.



(To convert µg/dL to nmol/L, multiply by 27.59.)

However, these changes were not significant and remained within normal limits.

The fact that most subjects demonstrate a decrease in mean cortisol concentrations following the diet intervention is interesting. Anderson et al. (1987) reported lowered cortisol concentrations in men in response to a 10-day, high CHO diet (70% CHO, 10% protein, 20% fat) but not to a high protein diet (35% CHO, 44% protein, 21% fat). Elevated testosterone and SHBG levels in conjunction with decreased CBG levels were also associated with the high CHO diet. Anderson suggests the ratio of CHO to protein in the diet represents an important factor in the regulation of these substances in the blood, either by altering the oxidative and reductive metabolism of steroids or by affecting the synthesis of their carrier proteins within the liver.

It has been suggested that low availability of certain amino acids within the circulation, may affect the synthesis of the neurotransmitter, serotonin; a substance known to regulate GnRH and gonadotropin release within the brain (Steiner et al. 1983). Tryptophan, an important precursor in the synthesis of serotonin within the brain is possibly increased in response to a carbohydrate-rich meal and the ensuing insulin response. Tryptophan competes with other amino acids such as valine, leucine and phenylalanine for uptake and transport within the blood. Thus, altering the ratio of protein to carbohydrate in the diet may change circulating concentrations of these amino acids, enhancing serotonin synthesis and affecting GnRH and gonadotropic release accordingly (L'anson et al. 1991).

In the present study, no significant correlation was found between changes in protein and CHO intake and changes in cortisol concentrations. However, the protein/CHO ratio was decreased (from approximately 14/54 to approximately 15/74) during the 5-day diet intervention. If steroidal metabolism is controlled by the protein/CHO ratio (Anderson et al. 1987), it could offer an explanation as to why reduced cortisol concentrations are displayed by the majority of subjects.

Conclusions

Results from this study preclude the importance of short-term dietary fat restriction in the development of reproductive dysfunction. Certain trends in the findings, including lowered serum cortisol levels and reduced luteal phase salivary progesterone concentrations, suggest that significant differences may have been detected if the length of the diet period extended. However, this is a matter for speculation. Due to the high palatability of fats and perceived early satiety associated with fat consumption, adherance to an extreme reduction in dietary fat is difficult for many people to maintain for an extended period of time (Rolls & Shide, 1992). On an anecdotal note, all subjects participating in this particular study reported relief upon completion of the 5-day, fat-restricted diet and were anticipating their first high-fat meal. Most felt a longer diet period would have been extremely difficult, if not impossible, to endure. Thus, subject compliance may have been compromised if a longer diet period had been instituted.

It is felt that the major fault inherent in this study was the failure to control the amount of change in dietary fat experienced by each subject. Change in dietary fat was in relation to each subject's normal dietary intake. Each subject varied in regards to their normal fat intake. Thus, % change in fat intake ranged from 15 - 28% (mean = 20 ± 1.4 %). This problem could have been overcome in 2 ways:

1. Placing all subjects on a control diet, identical in calorie and macronutrient content, one month prior to the low-fat diet intervention.

Hormonal comparisons would then be made between this control diet month and the fat-reduced diet month.

2. Presetting the % change in fat to a specific value (perhaps 20%) and changing the fat content of the low-fat diet intervention accordingly, in relation to each subject's normal dietary fat intake. Thus, the individual normally consuming 40% fat daily, would consume 20% fat during the diet-intervention period, while the individual normally consuming 30%, would consume 10% fat daily. Individuals who consume less than 30% fat on average would be excluded from the study.

High dietary fiber intake has been associated with delayed menarche (Hughes & Jones 1985) and changes in estrogen metabolism (Adlercreutz et al. 1987, Rose et al. 1991, Feng et al. 1993). It is possible that the failure to control fiber intake in this study, may have confounded results. However, mean fiber intake during the baseline was 19.4 grams or approximately 3% of total calorie intake (considering that fiber is composed of carbohydrate). Mean fiber intake during the fat-reduced diet was 33.0 grams or approximately 6% of energy intake. In terms of percentage of energy intake, this represents only a 3% change. It is questionable as to whether this would be enough of a change to stress the reproductive system.

Comparison of fiber intake between individuals shows that the change in fiber ranged from an average of 3 grams/day (10% change) to an average of 24 grams/day (65%). This wide variation was a result of individual differences in normal dietary intake as well as inconsistencies in the menus made up for each individual. In order to make the diet as agreeable to each subject as possible, the food items on the menu were adjusted to meet individuals' personal likes and dislikes. As well, the higher baseline caloric intakes of some individuals, in combination with a baseline high fat/low fiber content, resulted in these individuals receiving larger increases in complex carbohydrates and fiber during the diet intervention period. The result was inconsistent changes in fiber intake between subjects. Again, this could have been controlled by either of the 2 methods mentioned above. Also, having a standard menu for all subjects would better control differences in nutrient intakes.

Recommendations for Further Research

Despite the inherent problems associated with this research, there is little evidence to suggest that short-term, fat-reduced diet interventions affect the hormonal milieu of the normal young woman. Trends in the results suggest that extending the length of the diet intervention may have resulted in changes of greater significance. Extreme reductions in dietary fat intake represent permanent lifestyle changes for some women. Thus, the effects of a longer reduced-fat diet intervention may be a more relevant question to pursue.

The fact that vegetarian populations display differences in reproductive function deserves further investigation. In light of the research done by Hill et al. (1984, 1986), it may be worthy to further research the importance of animal protein in the diet. Focusing on specific amino acids and protein/carbohydrate ratios may be of particular interest. Equally important, the issue of high fiber consumption and steroidal metabolism should be settled.

In order to accurately assess the impact of each dietary constituent upon the reproductive axis, it is necessary to maintain tight control over consumption of all others cited as possible contributing factors. Longer study periods would be optimal, but obtaining compliance from human subjects for extended periods is a major problem. It may be more beneficial to further pursue this question through the use of animal models. The fact that dieting has been implicated in the genesis of secondary amenorrhea and the development of osteoporosis in young women, underlines the importance of further researching this matter.

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APPENDICES

Appendix 1

SUBJECT INFORMATION

DATE:____________________

NAME: ________________________________

ADDRESS: ______________________________

____

____

TELEPHONE:____________________

DATE OF BIRTH: ________________________ AGE:__________________

HEIGHT:__________________ (cm WEIGHT:__________________(kg)

__________________ (inches __________________(lbs)

Have you experienced a significant weight loss or gain in the last 12 months?

NO______________ YES______________

If yes, please specify amount of loss or gain and time involved ______________________

____

****

PHYSICAL ACTIVITY INFORMATION

1. Are you currently involved in a regular routine of physical activity or exercise?

(Minimum of 3 times/week for at least 4 months)

NO ____________

YES ____________

If yes, describe (activity, duration, frequency) ______________________________

____

____

____

MENSTRUAL CYCLE INFORMATION

2. At what age did you have your first menstrual period? ________________________

3. Have you taken oral contraceptive pills within the last 6 months?

NO ____________

YES ____________

4. List any medications you are currently taking ____

____

____

5. Is your current menstrual cycle regular? YES ___________ NO __________

6. How many periods do you usually have in a year? ____________________________

7. What is the interval of days between menstrual cycles? Indicate the number of days between day 1 (onset of flow) of a period and day 1 of subsequent period.

____

8. On average, how many days does your period last? ________________________________

9. Date of last menstrual period ___________________________________

10. The following is a list of symptoms that may precede or accompany a period. Place an "X" beside those symptoms that occur regularly.

Abdominal cramps ____________

Lower back pain ____________

Swollen breasts ____________

Water retention ____________

Decreased strength ____________

Headache ____________

Nausea ____________

Depression ____________

Increased Appetite ____________

Decreased Appetite ____________

Others (specify) ____

Appendix 2

Protocol for Measurement of Aerobic Fitness

Test purpose and procedure are explained to the subject. Consent is collected.

Height and weight are measured.

ECG electrodes are applied and attached to ECG monitor.

Bicycle seat and handlebars are adjusted according to subject height and comfort.

Headgear and mouthpiece are fitted on subject and attached to metabolic measurement cart (Horizon).

Subject is allowed a 3 minute warm-up with no resistance applied.

Subject pedals at a cadence of 60 RPM throughout test.

Cycling begins at a resistance of 0.5 Kp.

Heart rate is monitored every minute.

Resistance is increased by 0.5 Kp every 2 minutes, until ventilatory threshold is reached, at which point resistance is increased by 0.5 Kp every 1 minute.

Subject will receive vigorous encouragement throughout test period.

Test is terminated when subject indicates inability to maintain current load.

Headgear is immediately removed and subject is allowed ample "cool-down" period at self-selected speed and resistance.

Appendix 3

7-DAY FOOD FREQUENCY QUESTIONNAIRE

Please indicate average number of servings of the following food items during a typical week:

An example is provided:

	How often?			
FOOD ITEM	# DAY	# WEEK	# MONTH	NEVER
Coffee/Tea (1 cup)	3			
Cheese (50 gm)		2		
Dining Out			1	
Vitamins				X

This person drinks coffee 3 times a day, eats cheese twice a week, dines out once a month and never uses a vitamin supplement.

---

a.) Dairy Products

	How often?			
FOOD ITEM	#/DAY	#/ WEEK	# MONTH	NEVER
Milk (1 cup) Ho / 2% / 1% / Sk (circle one)				
Cheese (50g)				
Yogurt (3/4 cup)				

b.) Meat and Alternatives

Poultry (100g)				
Fish (100g)				
Meat (100g)				
egg (1)				
other (peanut butter, tofu, beans)				

c.) Grain Products

Bread (1) or Bun/Pita (1/2) white / wwheat (circle one)				
Cereal (3/4 cup)				
Pasta/Rice (1/2 cup)				

d.) Fruit

1 med serving or 1/2 cup juice				

Appendix 3

7-DAY FOOD FREQUENCY QUESTIONNAIRE

e.) Vegetables

FOOD ITEM	#/DAY	#/ WEEK	# MONTH	NEVER
1/2 cup or salad-1 cup				

f.) Fats

Marg/Butter/Mayo (1tsp)				
Salad oil/Dairy creamer (1tbsp)				

g.) Other

Desserts/Sweets				
Alcohol (12 oz beer, 1.5 oz. liquor, 4 oz wine)				
Soft Drinks (11.5 oz)				
Coffee/Tea (1 cup)				
Vitamin Supplements				
Dining Out				

Please indicate any food restrictions: ______________________

____

____

Please indicate any food likes/dislikes: ______________________

____

____

Please indicate any food allergies: ______________________

____

____

Appendix 4

INSTRUCTIONS FOR SALIVA COLLECTION

COLLECTION DAYS:

Begin collection on Day 12 of the menstrual cycle, and continue until the onset of the next menstruation. This should equal approximately 18-20 collection days. Collection will occur during 2 menstrual cycles.

TIME OF COLLECTION:

Saliva should be collected in the AM, immediately upon waking and prior to eating or brushing the teeth.

METHOD OF COLLECTION:

1.) Rinse mouth vigorously with plain water. Wait 5 minutes.

2.) Open stopper, remove cotton swab and place in mouth. Avoid handling swab if possible.

3.) Chew cotton swab for 60 seconds.

4.) Gently expectorate cotton swab into the vial and close with the stopper.

5.) Place vial into plastic bag (provided), place inside cardboard box. Store in freezer.

6.) All vials will be collected at the end of the menstrual cycle.

N.B. All vials will be numbered and it is extremely important that they are used in the correct sequence. If, for some reason a collection is missed, please make note of this, disregard that particular vial and continue the collection the next day maintaining the correct sequence. Thank-you for your time.

Appendix 5

Blood Sampling Protocol

7:30 a.m. Patient will register at Emergency/Admitting and bring name plate and admitting papers to Unit 5E.

8:00 a.m. Indwelling intravenous of normal saline (TKVO) started by Registered Nurse.

9:00 a.m. Commence blood sampling at 10 minute intervals for 8 hours (until 5:00 p.m.).

Details of sampling:

i) Close IV line
ii) Withdraw saline through line with syringe (5cc)
iii) Collect 2 ml sample in second syringe (5cc)
iv) Transfer blood carefully to (3ml) red top vacutainer
v) Flush line and reopen IV TKVO

5:00 p.m. Indwelling catheter removed - pressure applied for 2-5 minutes and bandaid applied.

Handling of Blood Samples

Blood samples **must** sit in vacutainer for at least 30 minutes but no more than 60 minutes prior to spinning.

Spin in chilled centrifuge for 10 minutes @ 4000 rpm (1000g).

Aliquot equally into 2 pre-labeled, polypropylene snap-cap storage vials (epindorphs).

Store in small white cardboard freezer boxes (labeled A & B) and leave in lab freezer.

Pack into cooler with dry ice and transfer to -80°C freezer (Physical Education).

Required Supplies

1 #22 intracath

1 Intravenous tubing & IV pole.

1000 ml normal saline

50 red top vacutainers (3 ml volume)

100 polypropylene snap-cap storage vials (epindorphs)

alcohol swabs, tape

2 white cardboard freezer boxes

Preparation for Sampling

Ensure availability of above supplies on unit.

Label 49 red top vacutainers with 10 minute time intervals.

Label 100 snap-cap storage vials (subject name, date, time, in duplicate A & B)

Label lid of white cardboard freezer box with subject name, date, day of cycle, "**Control**" or "**Post-Diet**", Harber/Sanderman & **A** or **B**.

Appendix 6

Radioimmunoassay for Luteinizing Hormone

LH Irma (Diagnostic Products Corporation, Intermedico, Markham, Ont) is an immunoradiometric assay in which LH in the sample is labeled with polyclonal anti-LH ^{125}I-tracer and immobilized to the wall of a polystyrene test tube treated with monoclonal anti-LH antibodies. Unbound ^{125}I-labeled anti-LH-antibodies are removed by washing. LH concentration in the sample is directly proportional to the radioactivity present in the tube. Assay precision, defined as the lowest detectable concentration from zero at the 95% confidence level, is reported as 0.15 mIU/ml.

Procedure

Blood samples were collected via intravenous catheter in untreated test tubes and allowed to coagulate. Samples were centrifuged at 4° C and aliquoted into 2 separate vials for storage at -80° C. All samples were assayed in duplicate. Samples were thawed at room temperature and mixed gently prior to assay.

1.) Set up standard curve. To increase precision at low concentrations, calibrators were diluted with zero calibrator to produce the following concentrations: 0, 0.75, 1.5, 3.75, 15 & 30 mIU/ml.

2.) Label all tubes in duplicate:

- 2 uncoated polystyrene tubes for total counts
- 12 coated tubes for standard curve
- 2 coated tubes for each control. In this case, 3 samples of unknown concentrations (low, intermediate and high) were assayed

-2 coated tubes for each subject sample

3.) Pipet 200 µl of each sample (calibrator, control or subject) into prepared tubes.

4.) Add 100 µl of ^{125}I LH ab to each tube.

5.) Shake for 1 hour on a rack shaker.

6.) Decant. Add 2 ml buffered wash solution. Wait 2 minutes and decant thoroughly.

7.) Count radioactivity for 1 minute in a gamma counter.

Calculation of Results

Using standard curve samples, percent bound (%bound/maximum binding) versus concentration is plotted in a log-log representation. Concentrations of subject and control samples are determined from the calibration curve by interpolation.

LH Pulse Analysis

LH pulse features were analyzed by a computer-assisted program (Monroe 1.1) using the Cluster Analysis method (Veldhuis & Johnson 1986). This algorithm uses a shifting window technique to compare adjacent nadir and peak clusters. Pooled *t* testing is used to judge the occurrence of significant increases or decreases within the data series. Nadir and peak cluster sizes and the pooled *t* statistic value are all specified by the operator. A 2 x 2 cluster size with a *t* stat of 1.89 yields a false-positive rate of ~ 4%. This is reduced to ~1% when a *t* stat of 2.92 is used. The program uses internal standard deviates calculated from the actual assay replicates making the program very sensitive to changes in intra-assay coefficient of variations. High assay precision will be reflected by lower coefficients of variation resulting in a greater number of pulse detections. The program determines the following parameters: pulse count, pulse interval, pulse amplitude, pulse area, mean nadir and mean measured level.

In order to acquire the most accurate determination of LH pulsatility, replicates were analyzed in 3 different ways: *t* stat 1.89, 1 x 3 cluster size, *t* stat 1.89, 3 x 3 cluster size, *t* stat 2.96, 2 x 3 cluster size. The results from the 3 determinations were combined with those obtained from eyeballing and the mean value calculated. From these results, time a (menstrual cycle one) was compared to time b (menstrual cycle two).

Intra and Interassay Coefficient of Variation

Each subject's samples were analyzed within the same assay reducing the importance of interassay (between) coefficients of variation. CV's from control samples were averaged over the 10 days and used to calculate the interassay CV. Intra-assay CV was calculated from the means of the CV from control duplicates plus every 10th set of duplicates (ie duplicates 10, 20, 30 .... 200). The intra- and interassay CV's for LH were 5.3% and 5.4% respectively.

Radioimmunoassay for Cortisol

Coat-a-Count Cortisol (DPC) is an example of a competive binding radioimmunoassay. Unknown quantities of cortisol (subject sample) compete with ^{125}I- labeled cortisol (known quantity) for antibodies immobilized on the wall of a polypropylene tube. The unlabeled and labeled cortisol then compete for the limited binding sites in proportion to their concentration. Thus, large quantities of unknown will result in lesser quantities of the labeled substance becoming bound. Comparison to a standard curve reveals the concentration of the unknown quantity. This is reflected as an inverse relationship, in that higher % bound of the known quantity means that lower concentrations of the unknown are present.

Procedure

Samples were collected, stored and thawed as described above.

1.) Prepare a standard curve. The following concentrations were used: 0, 1, 5, 10, 20 & 50 µg/dL.

2.) Label all tubes in duplicate:

- 2 plain tubes for total counts
- 2 plain tubes for nonspecific binding
- 12 coated tubes for standard curve
- control samples (low, intermediate, high)
- subject samples

3.) Pipet 25 µl of either calibrator, control or subject samples into prepared tubes.

4.) Add 1.0 ml of ^{125}I-cortisol to each tube. Vortex.

5.) Incubate at 37° C for 45 minutes.

6.) Decant thoroughly.

7.) Count for 1 minute in a gamma counter.

Calculation of Results

Using standard curve samples, percent bound (%bound/maximum binding) versus concentration is plotted in a linear-log representation. Concentrations of subject and control samples are determined from the calibration curve by interpolation.

Intra- and Interassay CV

Intra- and interassay CV's, calculated as described above, were 7.3 and 4.6 respectively. Lower detection limit for Coat-a-Count Cortisol is reported as ~0.2 µg/dL.

Progesterone Radioimmunoassay Procedure

Coat-A-Count Progesterone (DPC) is an example of a competitive binding assay in which unknown quantities of progesterone present in subject samples compete for binding sites with ^{125}I-labeled progesterone. Progesterone antibodies are fixed on the test-tube wall. Following decantation, the concentration of antibody-bound radioactive progesterone is measured. Concentration of progesterone present in the subject sample is inversely related to value obtained.

A major disadvantage to radioimmunoassay measurement of salivary progesterone is the need for solvent extraction. Extraction increases the concentration of progesterone in the sample and eliminates extraneous compounds which may interfere with progesterone binding (Read 1989). A direct method of radioimmunoassay for progesterone has been described (Bourque et al. 1986). Results from this study were in agreement with previously published values and the procedure is considered a satisfactory method for analyzing progesterone profiles. The low end of sensitivity for Coat-A-Count Progesterone is reported as 0.03 ng/ml (30 pg/ml). All subjects' salivary concentrations for this investigation were above this value.

Procedure

Saliva samples were thawed overnight at 4° C. To extract saliva from the cotton wad, salivettes were centriguged at 1,000 G for 2 minutes.

1.) Set up the standard curve. To increase the sensitivity of the standard curve at low concentrations, calibrators were diluted to produce the following concentrations: 0, 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0 ng/ml.

2.) Label all tubes in duplicate.

- 2 plain tubes for total count
- 2 plain tubes for nonspecific binding
- 16 coated tubes for standard curve
- 4 coated tubes for control samples (low, intermediate). In this case, the high control was deemed unnecessary, because it would have exceeded the range of the standard curve.
- coated subject sample tubes

3.) Pipet 100 µl of calibrators, control samples or subject samples into labeled tubes.

4.) Add 100 µl of ^{125}I-progesterone to each tube. Vortex.

5.) Incubate for 3 hours at room temperature.

6.) Decant thoroughly.

7.) Count for 1 minute in a gamma counter.

Calculation of Results

Using standard curve samples, percent bound (%bound/maximum binding) versus concentration is plotted in a linear-log representation. Concentrations of subject and control samples are determined from the calibration curve by interpolation.

Intra- and Interassay CV

Intra- and interassay CV's, calculated as described above were 7.7 % and 4.9 % respectively.

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Appendix 7

Example of Diet Plan

Day 1 & 4 Diet Plan -2000 Kcal

ITEM	QUAN	E	PRO	FAT	CHO
BREAKFAST					
WW Toast	1 sl	59	2.3	1.1	10.9
Rice krispies	1 cup	112	1.9	0.2	25
Milk Skim	1.5 cup	135	12.6	0.6	17.9
orange juice	1 cup	104	1.6	0.4	24.6
Jam	1Tbsp	54	0.1	0.0	14.0
Total		479	18.5	2.3	92.4
AM SNACK					
arrowroot	2	48	0.8	1.8	8.6
orange	1	65	1.4	0.1	16.3
Total		113	2.2	1.9	24.9
LUNCH					
Beef sandwich					
WW bread	2 sl	118	4.6	2.2	21.8
Roast beef	50g	76	11.6	2.6	1.0
Mayo	.5 tsp	32	0.1	1.9	0
salad	1 cup	80	1.5	0.3	18.1
grape juice	1 cup	155	1.4	0.2	38
Total		461	19.0	7.2	78.9
PM SNACK					
NF Plain Yogurt	1 cup	144	12.0	3.6	16.0
apple	1	81	.3	.5	21.1
Total		225	12.3	4.1	37.1
SUPPER					
Beef stew	1 T2	155	14.6	4.6	14.0
WW Bun	1	72	2.8	0.8	14.6
Rice	1 T2	111	2	0.1	24.8
grape juice	1 cup	155	1.4	0.2	38
Banana	1 med	105	1.2	0.6	26.7
Total		598	22	6.3	118.1
HS SNACK					
Melba toast	4	154	4.8	1.2	30.2
Total		154	4.8	1.2	30.2
TOTAL		**2029**	**79**	**23**	**381.6**
%			**16%**	**10%**	**75%**

Appendix 8

UNIVERSITY OF ALBERTA

Department of Physical Education & Sport Studies

SUBJECT CONSENT TO PARTICIPATE IN A RESEARCH STUDY

The Effect of Dietary Fat on Luteinizing Hormone and Cortisol Levels

INVESTIGATORS

Dr. V.J. Harber, Dr. D.C.Cumming, B. Marriage,
A. Sanderman

****

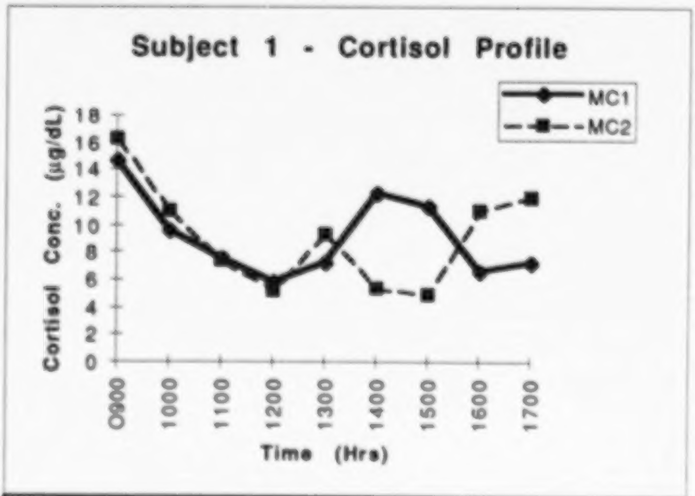
The study has been satisfactorily explained to me by Dr. Harber/Dr. Cumming/B. Marriage/A. Sanderman, or their designate. I understand the necessity for the procedures outlined in the **Study Information** sheet. I know that I may contact the person designated on this form, if I have further questions either now or in the future. I have been informed of the possible benefits of joining the research study, as well as the possible risks and discomforts. I understand that there will be no cost to me for study-related visits. I have been assured that the information obtained from my participation in this study may be published in medical reports, but that personal records will be kept confidential. I understand that I am free to withdraw without prejudice from the study at any time. I understand that I will be promptly informed of any findings which may develop during the research period that may affect my willingness to continue participating in the study. I understand that copies of the **Study Information sheet**, and the signed **Consent Form** will be given to me to keep.

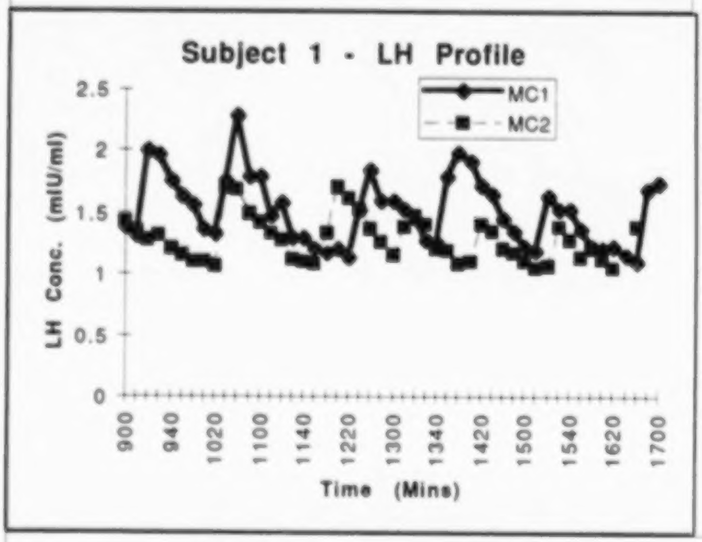
Subject name (print)	Subject signature & date
Witness name (print)	Witness signature & date
Investigator name (print)	Investigator signature & date

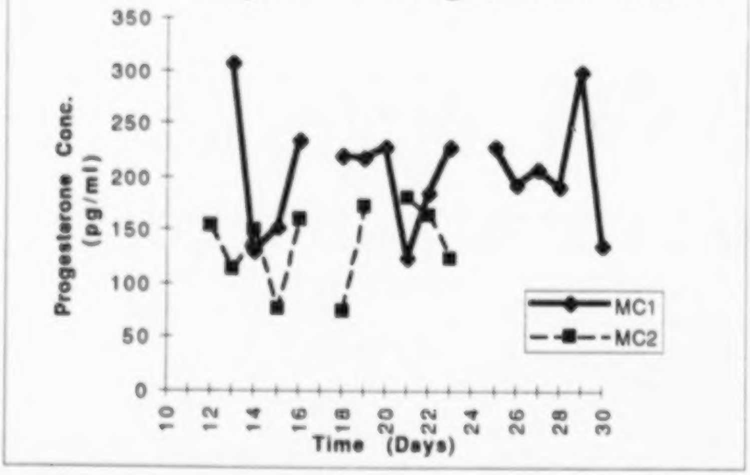
QUESTIONS OR CONCERNS MAY BE DIRECTED TO DR. HARBER AT 492-0581, BARB MARRAIGE AT 492-6005 OR ALEX SANDERMAN AT 481-0576.

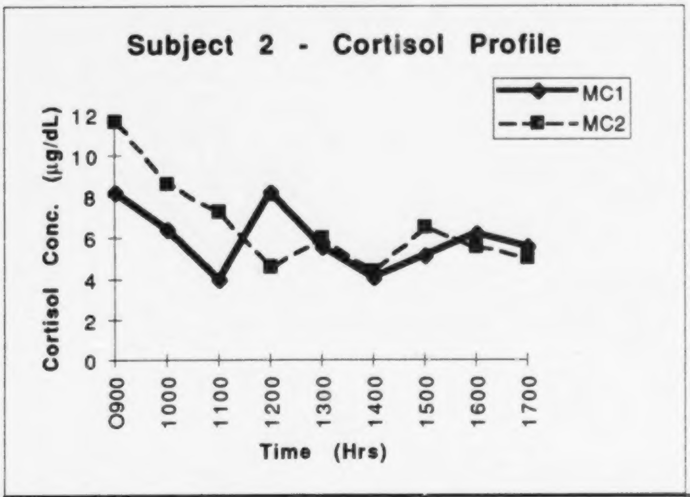
Appendix 9

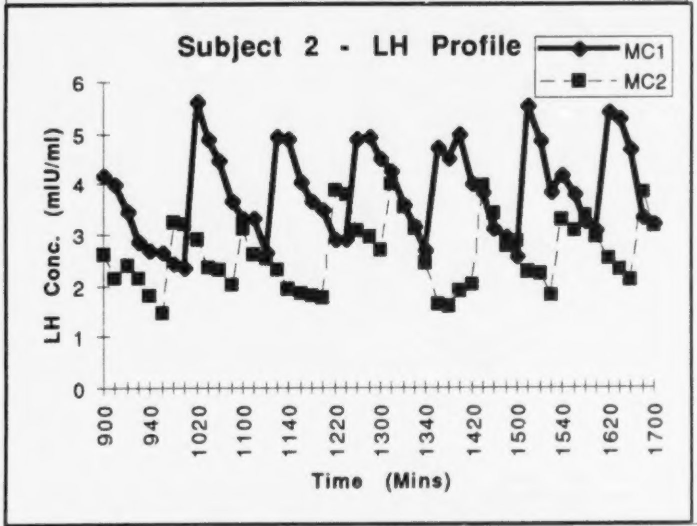
Subject Hormone Profiles

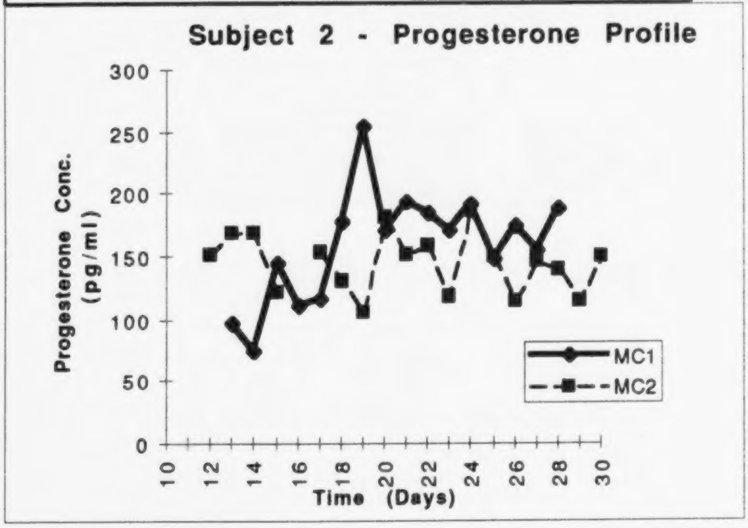

Subject 1 - Cortisol Profile
MC1
MC2
Cortisol Conc. (µg/dL)
0
2
4
6
8
10
12
14
16
18
0900
1000
1100
1200
1300
1400
1500
1600
1700
Time (Hrs)

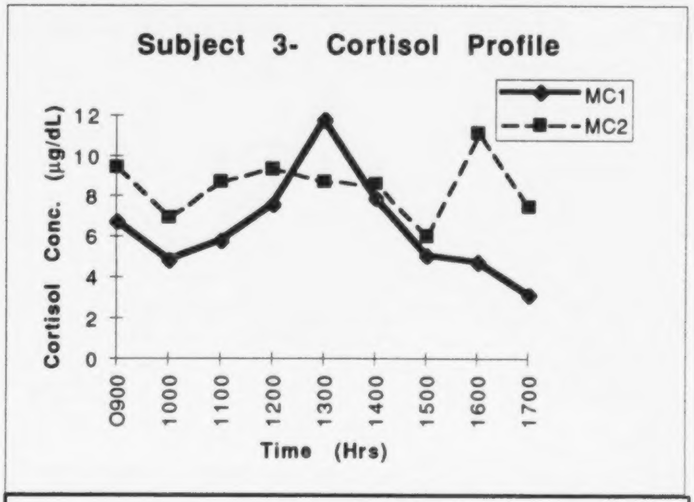

Subject 1 - LH Profile
MC1
MC2
LH Conc. (mIU/ml)
0
0.5
1
1.5
2
2.5
900
940
1020
1100
1140
1220
1300
1340
1420
1500
1540
1620
1700
Time (Mins)

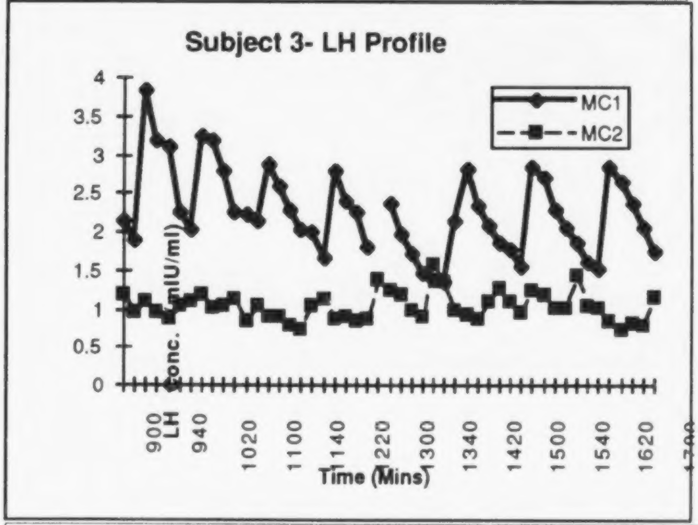

Subject 1 - Progesterone Profile
Progesterone Conc. (pg/ml)
0
50
100
150
200
250
300
350
10
12
14
16
18
20
22
24
26
28
30
Time (Days)
MC1
MC2

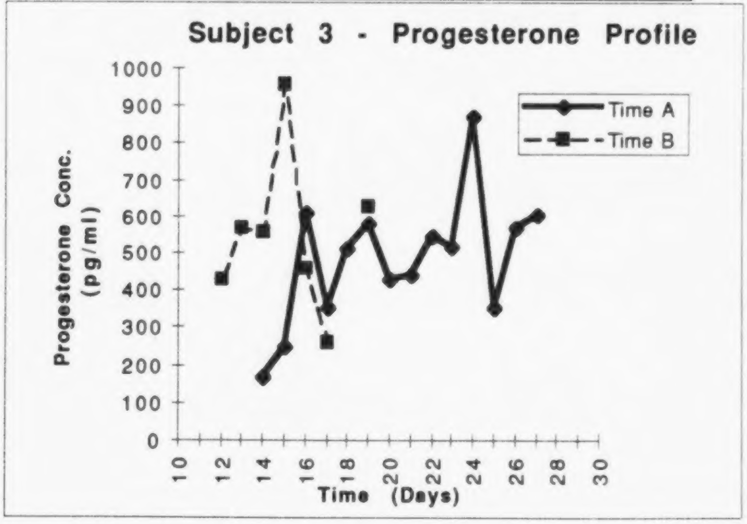

Subject 2 - Cortisol Profile
MC1
MC2
Cortisol Conc. (µg/dL)
12
10
8
6
4
2
0
O900
1000
1100
1200
1300
1400
1500
1600
1700
Time (Hrs)

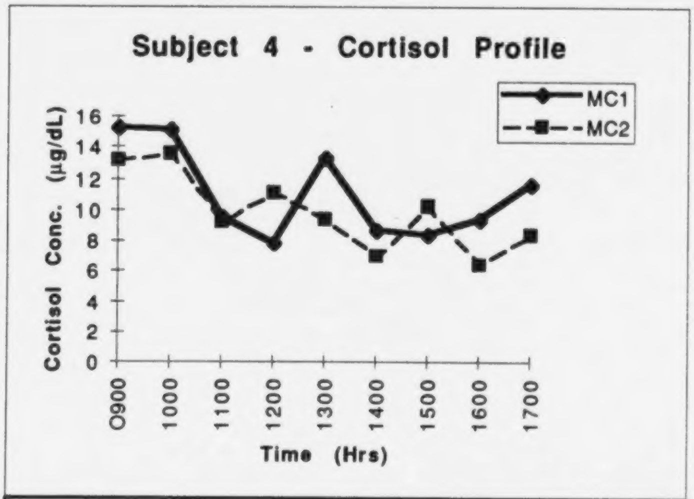

Subject 2 - LH Profile
MC1
MC2
LH Conc. (mIU/ml)
6
5
4
3
2
1
0
900
940
1020
1100
1140
1220
1300
1340
1420
1500
1540
1620
1700
Time (Mins)

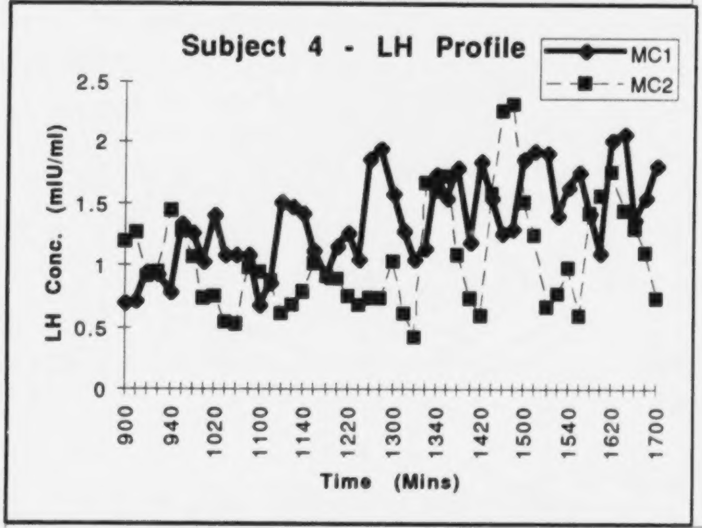

Subject 2 - Progesterone Profile
Progesterone Conc. (pg/ml)
300
250
200
150
100
50
0
10
12
14
16
18
20
22
24
26
28
30
Time (Days)
MC1
MC2

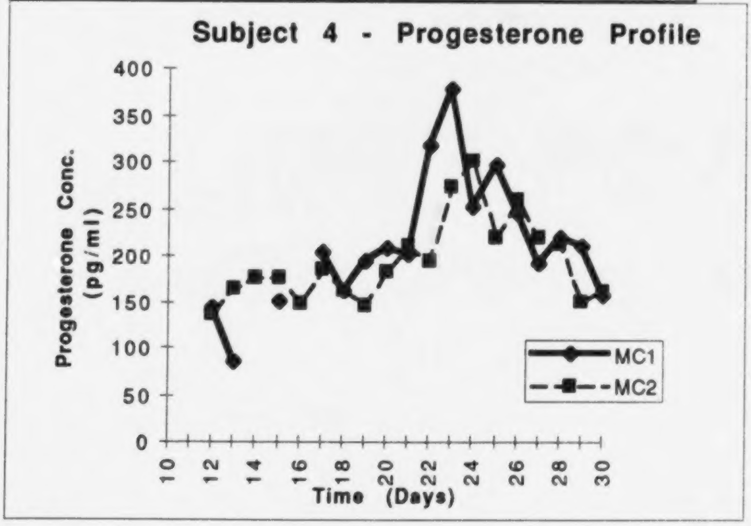

Subject 3- Cortisol Profile
MC1
MC2
Cortisol Conc. (µg/dL)
12
10
8
6
4
2
0
O900
1000
1100
1200
1300
1400
1500
1600
1700
Time (Hrs)

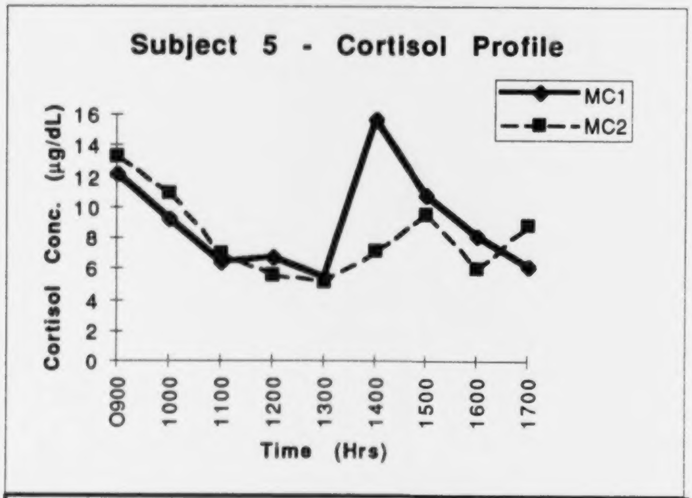

Subject 3- LH Profile
MC1
MC2
4
3.5
3
2.5
2
1.5
1
0.5
0
LH Conc. mIU/ml)
900
940
1020
1100
1140
1220
1300
1340
1420
1500
1540
1620
Time (Mins)

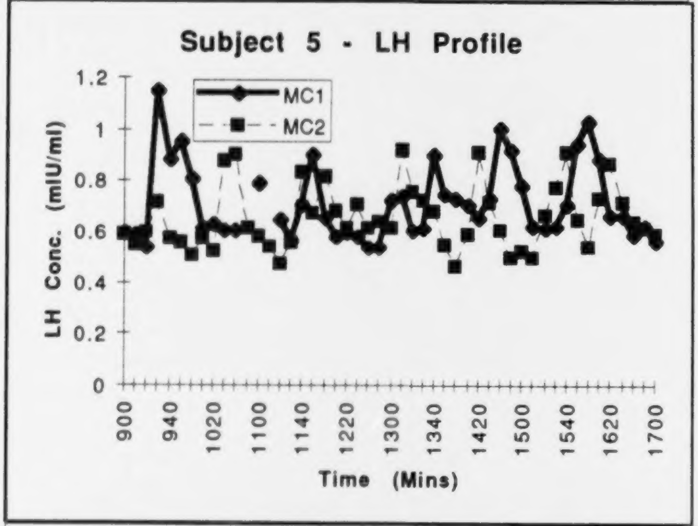

Subject 3 - Progesterone Profile
Time A
Time B
Progesterone Conc. (pg/ml)
1000
900
800
700
600
500
400
300
200
100
0
10
12
14
16
18
20
22
24
26
28
30
Time (Days)

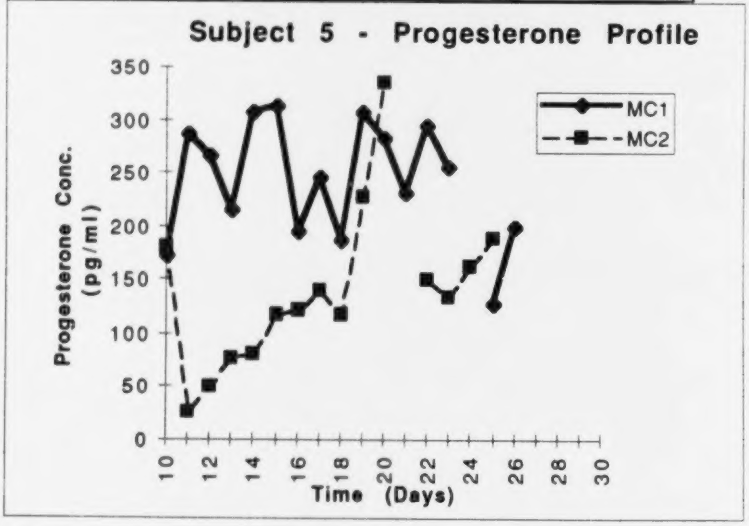

Subject 4 - Cortisol Profile

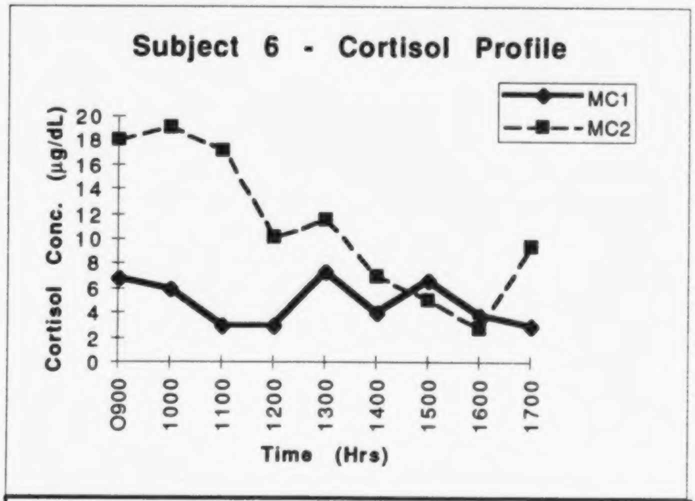

Subject 4 - LH Profile

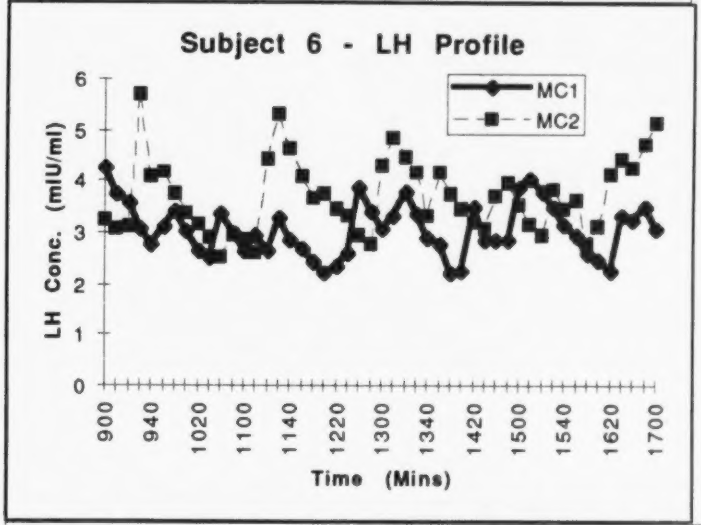

Subject 4 - Progesterone Profile

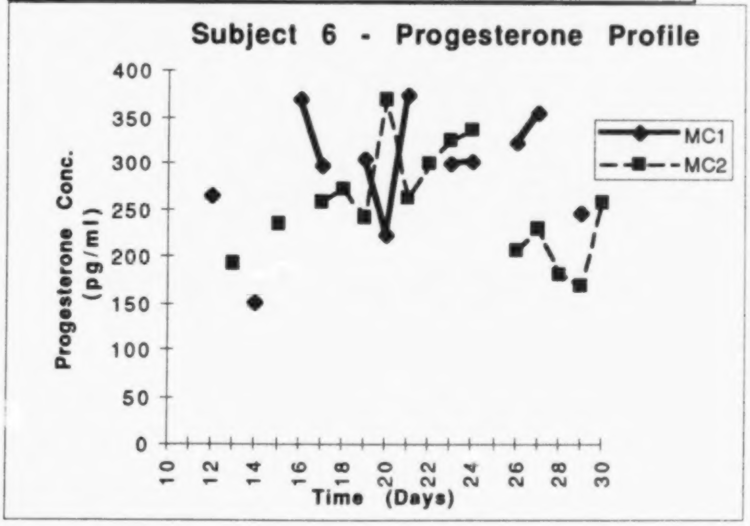

Subject 5 - Cortisol Profile
MC1
MC2
Cortisol Conc. (µg/dL)
16
14
12
10
8
6
4
2
0
O900
1000
1100
1200
1300
1400
1500
1600
1700
Time (Hrs)

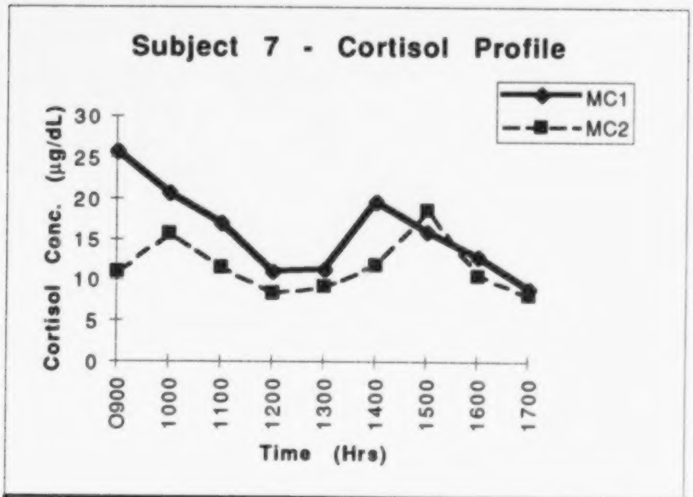

Subject 5 - LH Profile
MC1
MC2
LH Conc. (mIU/ml)
1.2
1
0.8
0.6
0.4
0.2
0
900
940
1020
1100
1140
1220
1300
1340
1420
1500
1540
1620
1700
Time (Mins)

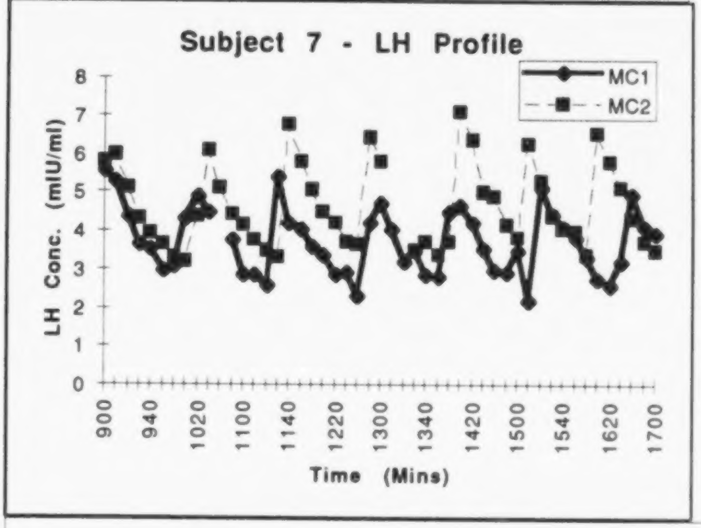

Subject 5 - Progesterone Profile
MC1
MC2
Progesterone Conc. (pg/ml)
350
300
250
200
150
100
50
0
10
12
14
16
18
20
22
24
26
28
30
Time (Days)

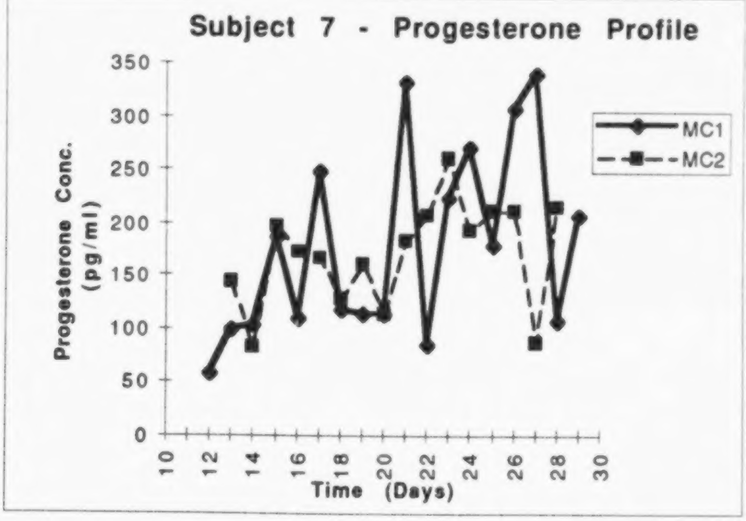

Subject 6 - Cortisol Profile
MC1
MC2
Cortisol Conc. (µg/dL)
0
2
4
6
8
10
12
14
16
18
20
O900
1000
1100
1200
1300
1400
1500
1600
1700
Time (Hrs)

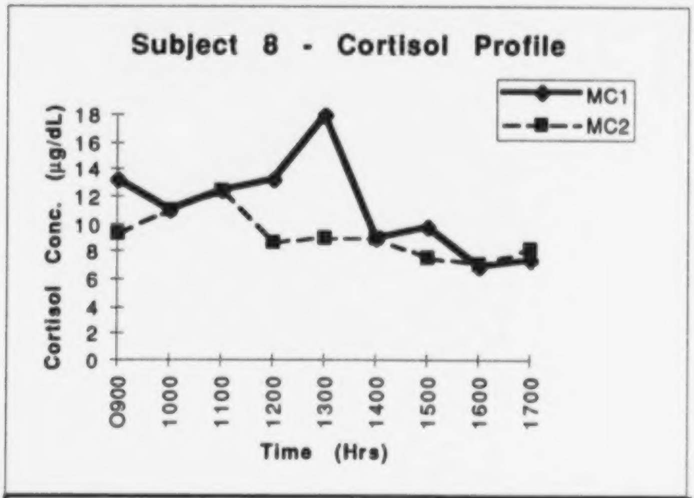

Subject 6 - LH Profile
MC1
MC2
LH Conc. (mIU/ml)
0
1
2
3
4
5
6
900
940
1020
1100
1140
1220
1300
1340
1420
1500
1540
1620
1700
Time (Mins)

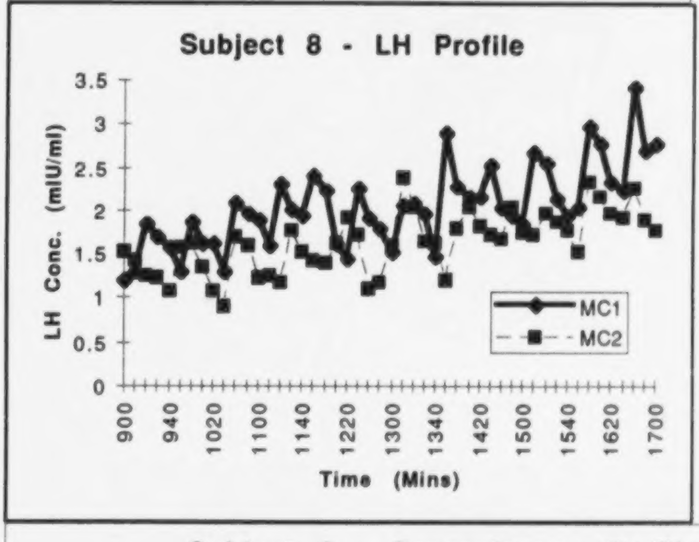

Subject 6 - Progesterone Profile
MC1
MC2
Progesterone Conc. (pg/ml)
0
50
100
150
200
250
300
350
400
10
12
14
16
18
20
22
24
26
28
30
Time (Days)

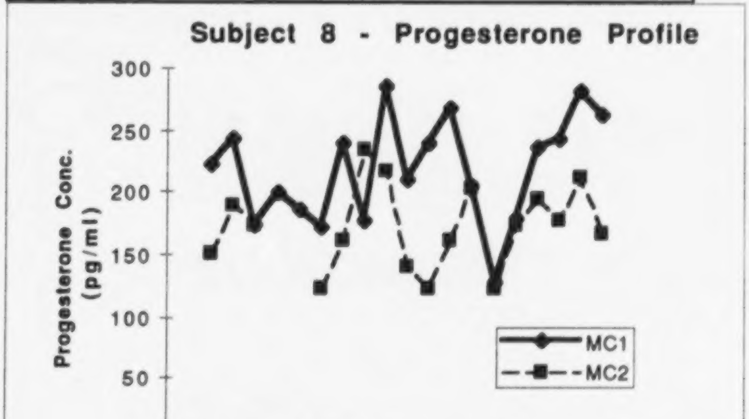

Subject 7 - Cortisol Profile
MC1
MC2
Cortisol Conc. (µg/dL)
0
5
10
15
20
25
30
O900
1000
1100
1200
1300
1400
1500
1600
1700
Time (Hrs)

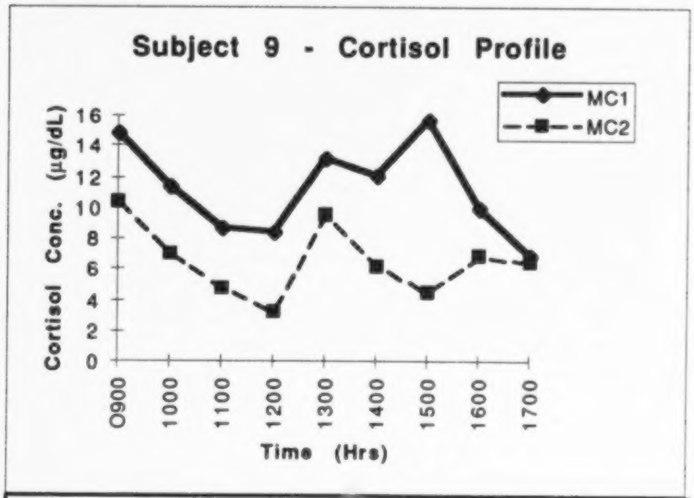

Subject 7 - LH Profile
MC1
MC2
LH Conc. (mIU/ml)
0
1
2
3
4
5
6
7
8
900
940
1020
1100
1140
1220
1300
1340
1420
1500
1540
1620
1700
Time (Mins)


Subject 7 - Progesterone Profile
MC1
MC2
Progesterone Conc. (pg/ml)
0
50
100
150
200
250
300
350
10
12
14
16
18
20
22
24
26
28
30
Time (Days)

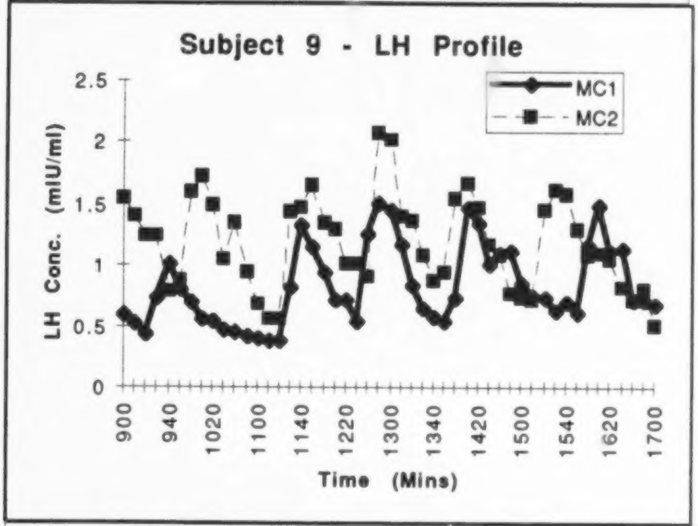

Subject 8 - Cortisol Profile
MC1
MC2
Cortisol Conc. (µg/dL)
18
16
14
12
10
8
6
4
2
0
O900
1000
1100
1200
1300
1400
1500
1600
1700
Time (Hrs)


Subject 8 - LH Profile
MC1
MC2
LH Conc. (mIU/ml)
3.5
3
2.5
2
1.5
1
0.5
0
900
940
1020
1100
1140
1220
1300
1340
1420
1500
1540
1620
1700
Time (Mins)

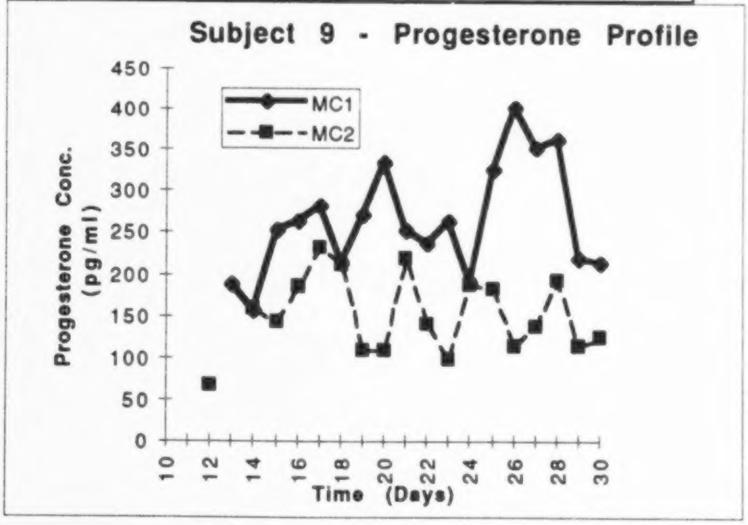

Subject 8 - Progesterone Profile
MC1
MC2
Progesterone Conc. (pg/ml)
300
250
200
150
100
50
0
10
12
14
16
18
20
22
24
26
28
30
Time (Days)



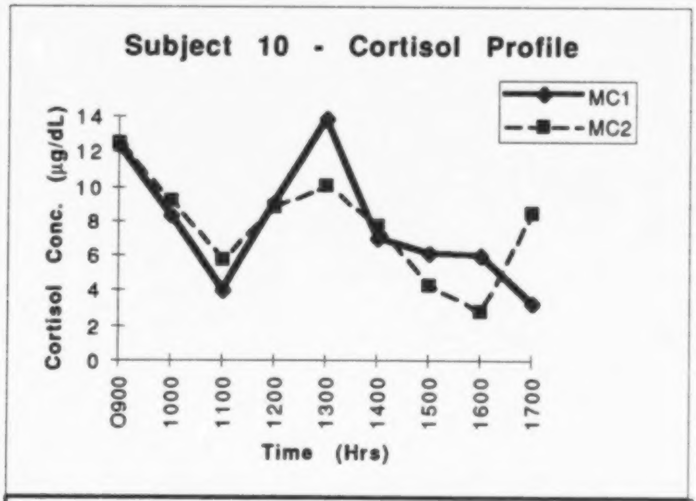
Subject 9 - Cortisol Profile



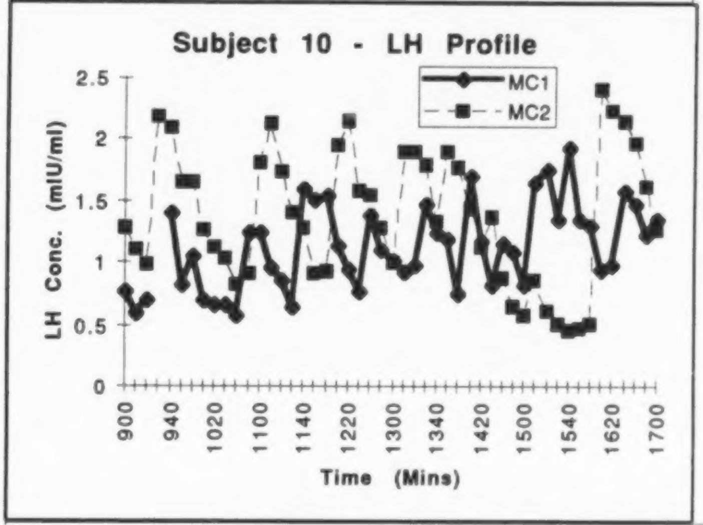
Subject 9 - LH Profile



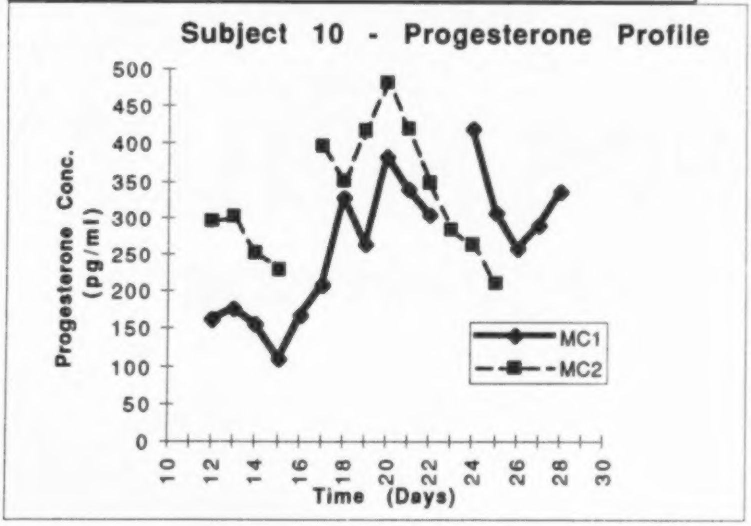
Subject 9 - Progesterone Profile



Subject 10 - Cortisol Profile



Subject 10 - LH Profile



Subject 10 - Progesterone Profile

Appendix 10a

Pearson Correlation Matrix (N = 10)

	SOS	BMI	ΔKcal	ΔPro	ΔCHO	ΔT Fat	ΔSat	ΔMono	ΔPoly	ΔCholes	ΔFiber	ΔX Cort	ΔC area	ΔX LH	ΔLH area	ΔLH Freq	ΔLH Amp
SOS	1.00	0.57	0.51	0.03	0.26	0.39	0.28	0.33	0.50	0.14	0.10	-0.51	-0.51	0.42	0.16	0.25	0.21
BMI	0.57	1.00	-0.12	0.24	-0.01	0.47	0.44	0.45	0.29	0.34	-0.04	-0.30	-0.30	0.43	0.08	0.50	0.23
ΔKcal	0.51	-0.12	1.00	0.08	0.25	0.17	-0.07	0.15	0.24	0.37	-0.24	-0.24	-0.24	-0.16	0.14	-0.27	0.00
ΔPro	0.03	0.24	0.08	1.00	0.34	-0.10	-0.17	-0.06	-0.18	0.18	0.12	0.42	0.42	-0.43	-0.44	0.65	-0.49
ΔCHO	0.26	-0.01	0.25	0.34	1.00	-0.56	-0.70	-0.52	-0.20	0.21	0.66	0.01	0.01	-0.26	-0.39	0.26	-0.50
ΔT Fat	0.39	0.47	0.17	-0.10	-0.56	1.00	0.85	0.98	0.83	0.86	-0.74	-0.58	-0.58	0.25	0.13	0.07	0.36
ΔSat	0.28	0.44	-0.07	-0.17	-0.70	0.85	1.00	0.78	0.73	0.18	-0.61	-0.36	-0.36	0.16	-0.04	0.25	0.23
ΔMono	0.33	0.45	0.15	-0.06	-0.52	0.98	0.78	1.00	0.83	0.50	-0.73	-0.64	-0.64	0.21	0.14	0.09	0.30
ΔPoly	0.50	0.29	0.24	-0.18	-0.20	0.83	0.73	0.83	1.00	0.45	-0.46	-0.76	-0.76	0.06	-0.13	0.21	0.05
ΔCholes	0.14	0.34	0.37	0.18	0.21	0.86	0.18	0.50	0.45	1.00	-0.35	-0.48	-0.48	0.04	-0.15	0.07	0.15
ΔFiber	0.10	-0.04	-0.24	0.12	0.66	-0.74	-0.61	-0.73	-0.46	-0.35	1.00	0.34	0.34	0.15	-0.07	0.27	-0.13
ΔX Cort	-0.51	-0.30	-0.24	0.42	0.01	-0.58	-0.36	-0.64	-0.76	-0.48	0.34	1.00	0.99	-0.23	-0.13	0.01	-0.10
ΔC area	-0.51	-0.30	-0.24	0.42	0.01	-0.58	-0.36	-0.64	-0.76	-0.48	0.34	0.99	1.00	-0.23	-0.13	0.01	-0.10
ΔX LH	0.42	0.43	-0.16	-0.43	-0.26	0.25	0.16	0.21	0.06	0.04	0.15	-0.23	-0.23	1.00	0.75	-0.31	0.89
ΔLH area	0.16	0.08	0.14	-0.44	-0.39	0.13	-0.04	0.14	-0.13	-0.15	-0.07	-0.13	-0.13	0.75	1.00	-0.61	0.83
ΔLH freq	0.25	0.50	-0.27	0.65	0.26	0.07	0.25	0.09	0.21	0.07	0.27	0.01	0.01	-0.31	-0.61	1.00	-0.59
ΔLH Amp	0.21	0.23	0.00	-0.29	-0.50	0.36	0.23	0.30	0.05	0.15	-0.13	-0.10	-0.10	0.89	0.83	-0.59	1.00

Appendix 10b

Significant Pearson r Correlations (N = 10)

	SOS	BMI	Kcal	Pro	CHO	T Fat	Sat	Mono	Poly	Choles	Fiber	X Cort	C area	X LH	LH area	LH Freq	LH Ampl
SOS	1.00																
BMI		1.00															
Kcal			1.00														
Pro				1.00													
CHO					1.00		-0.70										
T Fat						1.00	0.85	0.98	0.83	0.86	-0.74						
Sat							1.00	0.78	0.73								
Mono								1.00	0.83		-0.73	-0.64	-0.64				
Poly									1.00			-0.76	-0.76				
Choles										1.00							
Fiber					0.66						1.00						
X Cort												1.00	0.99				
C area													1.00				
X LH														1.00	0.75		
LH area															1.00		
LH freq				0.65											-0.61	1.00	
LH Ampl														0.89	0.83		1.00

$p < 0.05$

Appendix 10c

Pearson r Correlations
Progesterone with Hormonal, Dietary and Body Composition Factors

N=7	SOS	BMI	ΔKcal	ΔPro	ΔCHO	ΔT Fat	ΔSat	ΔMono	ΔPoly	ΔCholes	ΔFiber	ΔX Cort	ΔC area	ΔLH X	ΔLH area	ΔLH Freq	ΔLH Ampl
Δ X Prog	0	*-0.85	0.64	0.61	0.14	-0.68	-0.66	-0.72	-0.43	-0.66	0.39	0.53	0.53	-0.03	0.54	-0.65	0.24
Δ Prog Area	0	*-0.85	0.64	0.61	0.14	-0.68	-0.66	-0.72	-0.43	-0.66	0.39	0.53	0.53	-0.03	0.54	-0.65	0.24

* significant $p < 0.05$